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SAMUEL HENRY ESSARY
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Professor Samuel Henry Essary passed away suddenly on April 28, 1935, from a heart attack. At the time of his death he was Botanist of the Tennessee Agricultural Experiment Station and had served the institution since 1904. His researches in the field of botany and plant pathology were extensive in character but thorough, and consequently were well known by professional workers, not only in these fields but also in allied groups. He was equally well known for his comprehensive knowledge of the flora and geography of the Southern Appalachians. By continuously applying his knowledge of scientific principles to practical phases of plant improvement, he made notable contributions to the agriculture of the Southeast.

Born in West Tennessee, September 11, 1870, a descendant of pioneer stock, he grew up in an environment of nature on the home farm and always retained his love for the outdoors and agriculture. Completing the elementary schools, he entered the University of Tennessee, graduating in 1897. From 1899 to 1902 he taught in LaGrange College in Missouri, and from 1902 to 1904 in Brenau College at Gainesville, Georgia. In 1904 he was appointed instructor in the Botany Department of the University of Tennessee, and Assistant Botanist of the Experiment Station. He took his Master's degree at the same institution in 1907, and also spent some time in study at the University of Wisconsin. From 1912 to 1919 he was Associate Botanist of the Experiment Station, and became Botanist in 1919.

Always a public spirited man, he belonged to numerous organizations. He took an active interest in Masonic affairs, being a member of Kerbela Temple A.A.O.N.M.S. of Knoxville, Tennessee, and was a member of the Baptist denomination. He was a fellow of the American Association for the Advancement of Science, a charter member of the American Phytopathological Society, a member of the American Genetics Association, American Society of Agronomy, Tennessee Academy of Science, and other local scientific societies, and belonged to Phi Kappa Phi, Alpha Zeta, and the S.A.E. social fraternity.

Early in his career he was associated with the late S. M. Bain in developing anthracnose-resistant clover and carried the work steadily forward after Professor Bain's death in 1918. Improving a native lespedeza by careful selection, he produced "Tennessee 76," a variety widely grown in Tennessee and other states. His Tennessee Red and Tennessee Pink tomatoes were developed for wilt resistance and thus filled a sorely felt want. He made many selections of special regional value and tested many crops and native plants for their relative merit and their possible economic value. Forage crops and legumes seemed to hold his first interest, although he worked successfully with reference to varietal adaptation and disease control in other groups. During the past several years he had done considerable work with the selection and breeding of cotton.

While a student he became interested in the extensive region now composing the Great Smoky Mountains National Park. At that time it was a vast wilderness of deep gorges and rugged peaks, heavily timbered and abounding in wild life of all kinds. Professor Essary worked untiringly for the preservation of these natural beauties, and many of his excellent and unusual photographs which were anonymously published throughout the world were used in calling the attention of the public to the scenic value of this area. He blazed many trails through this virgin forest that others might follow safely, and spent many days botanizing in the region. His amiability and his desirability as a comrade in the woods can be vouched for by the many people who have spent days and nights with him over hard and tiring trails. His botanical collections for 40 years were very extensive and his intimate knowledge of the plants of the region was unsurpassed, due largely to his ability to cover great distances and his unusual powers of observation.

Usually called "Professor" Essary, he officially taught only a short while, but devoted many hours each day to research. Although a very industrious man, he always found time to help others with their particular problems and influenced numerous students to take up the field of Botany. Of an exceedingly kindly disposition and indifferent to his own personal wants and advancement, his greatest pleasure was to see the science for which he had devoted his own life advanced by the accomplishments of those younger men in whom he was especially interested.

BIBLIOGRAPHY

1. BAIN, S. M., and S. H. ESSARY. A preliminary note on clover diseases in Tennessee. Science (n. s.) **22**: 503. 1905.
2. ————, and ————. A new anthracnose of alfalfa and red clover. Jour. Mycol. **12**: 192–193. 1906.
3. ————, and ————. Selection for disease-resistant clover. Tennessee Agr. Expt. Sta. Bull. 75 (19, No. 1). 10 pp. 1906.

4. BAIN, S. M., and S. H. ESSARY. Four years' results in selection for a disease-resistant clover. Science (n. s.) 31: 756. 1910.
5. ESSARY, S. H. Notes on tomato diseases with results of selection for resistance. Tennessee Agr. Expt. Sta. Bull. 95. 12 pp. 1912.
6. ————————. Lespedeza, or Japan clover. Tennessee Agr. Expt. Sta. Press Bull. 34. [2] pp. 1914.
7. ————————. Sweet potato rots in Tennessee. Tennessee Agr. Expt. Sta. Press Bull. 41. [2] pp. 1915.
8. ————————. Report. Tennessee Agr. Expt. Sta. Rept. **31** (1918): 7–9; **32** (1919): 9–11; **33** (1920): 15–16.
9. ————————. Lespedeza (Japan clover). Tennessee Agr. Expt. Sta. Bull. 123. 28 pp. 1921.
10. MOOERS, C. A., and S. H. ESSARY. Agronomy. [Field crops work in Tennessee.] Tennessee Agr. Expt. Sta. Rept. **36** (1923): 19–23.
11. ESSARY, S. H. Tomatoes. Tennessee Agr. Expt. Sta. Rept. **40** (1927): 29.
12 ————————. Two important tomato diseases and their control. Tennessee Agr. Expt. Sta. Circ. 8: [4] pp. 1927.
13. ————————. Control of dodder in Lespedeza. Tennessee Agr. Expt. Sta. Circ. 22. [2] pp. 1928.
14. ————————. Three new varieties of Lespedeza. Tennessee Agr. Expt. Sta. Circ. 30. [3] pp. 1930.
15. ————————. Some common tomato diseases and their control. Tennessee Agr. Expt. Sta. Circ. 31. [4] pp. 1930.
16. ————————. Tomato diseases. Tennessee State Hort. Soc. Proc. **25** (1930): 93–96.
17. ————————. Select varieties of cotton. Tennessee Agr. Expt. Sta. Circ. **47**. [3] pp. 1934.

THE EFFECT OF CERTAIN CULTURAL AND HANDLING PRACTICES ON THE RESISTANCE OF APPLES TO PENICILLIUM EXPANSUM[1]

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The success of the Washington apple industry is due in part to its practice of placing on the market a constant supply of uniformly high quality fruit in convenient containers. Among the important factors of culture and handling that determine the quality and condition of fruit are the application of orchard fertilizers, the time of harvest, and the length of the storage period. To maintain a high yield the application of fertilizer has become standard practice. Since grade of apples is determined largely by the color, they are frequently left on the tree past prime maturity in order to meet the requirements for extra-fancy fruit. The commercial crop is held in cold storage for protracted periods, approximately half being stored locally and half at eastern terminals. The storage life of the three principal varieties of the State is as follows: the Jonathan crop is consumed largely by the last of January, the Delicious by March 1, and Winesap apples are available until about July 1. Fungous decay is augmented by this prolonged storage, and assumes great importance because of the high unit value of the crop and the necessity of preserving a favorable reputation. It is recognized by the members of the industry that there is a distinct correlation in a given variety between the length of the storage period and the percentage of decay.

Blue-mold decay, caused by *Penicillium expansum* Link, is the most important storage rot of apples. A conservative estimate[4] places the annual loss for the apples *actually decayed* by this organism in the Washington crop at about $448,000. The accompanying losses from such items as cost of repacking, lowering of price level, and dumping of fruit are far greater, but are not assessable.

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[3] The writers wish to express their appreciation of the cooperation of Mr. F. L. Overley, Associate Horticulturist, Wenatchee, in obtaining the fruit for these studies, and of the assistance of Mr. Otto Schnellhardt during the investigations.

[4] Computed from the average annual value ($27,988,400) of the commercial apple crop for the 5-year period, 1925–1929 (30), and the average 1.6 per cent of fruit that showed blue-mold decay in the 6.3 per cent of the total car shipments inspected at eastern terminals in the same period (3, 15, 28).

Penicillium expansum is able to infect apples through injuries of the epidermis and through lenticels, the calyx basin and canal, the stem, and necrotic areas. With proper handling of the varieties grown in Washington only the first two infection courts are of commercial importance. The ability of the fruit to withstand infection at lenticels, the stem, or the calyx basin or canal may be considered as external resistance. After infection has occurred, the rate of advance of the decayed area is influenced by the congeniality of the tissue as a substratum (*i.e.*, the degree of internal resistance). Much of the published work on resistance of apples to decay has dealt with the internal type, but some papers (3, 4, 6, 19, 15, 22, 31) have considered external resistance.

The purpose of these investigations was to determine the effect of application of orchard fertilizers, of maturity at the time of picking, of the length of the storage period, and of fruit variety on the incidence of lenticel infections and the rate of advance of the blue-mold decay of apples.

MATERIALS AND METHODS

The fruit used in these studies was obtained from the Experiment Station orchard in Wenatchee. It was sorted in the orchard for size (ranged from 113 to 150) and extra-fancy grade, only fruit free from injuries of the epidermis being used. It was packed without being washed, shipped at once to Pullman, and placed in cold storage ($0° \pm 0.3°$ C., unless otherwise specified).

The following method of inoculation of punctures was used in the series for determination of internal resistance. The end of a stick was cut so as to leave a peg projecting from the shoulder formed by the cut end; this tool gave artificial stem punctures in the fruit uniformly 4 mm. in diameter and of the same depth. Each apple was punctured at 3 points equidistant about its greatest circumference by this simple wooden device. The fruit was then immersed in an aqueous suspension of spores of the strain of *Penicillium expansum* employed in previous work (4, 5). Approximately the same amount of inoculum was employed each time in a uniform amount of water, and the fruit was rotated in it for a uniform period of time. The apples were then wrapped and packed in the usual way. Each spore suspension was made up as needed and was used for the inoculation of a single box of fruit.

Most of the studies on internal resistance of apples to fungous decay have been conducted by careful inoculation of a comparatively few specimens. Therefore, a justification should be given for the simple and rapid, though somewhat crude, method described above. The major objections to such a procedure are the possibility of contamination and the lack of complete uniformity of the amount of inoculum. Due to the characteristic

inhibitory action of *Penicillium expansum* on other apple-rotting fungi and its tolerance of their staling products (23), its relatively rapid growth in apples at cold storage temperatures, the high population of spores employed, and the fact that some fungi do not cause decay at 0° C., there was very little contamination in the series. In the 9294 punctures so inoculated only 23 (0.25 per cent) showed contamination. Such a condition is in line with the findings of Machacek (23) in the commercial storages of Montreal and of the writers in the commercial storages of Washington. The principal contaminant encountered in these studies was *Botrytis cinerea* Pers. Apples infected by organisms other than blue mold were not entered in the tabulated data. The same technique will not apply to other apple-rotting fungi. For example, a similar lot of fruit immersed in a suspension of spores of *Gloeosporium perennans* Zeller and Childs gave almost complete infection with *Penicillium expansum* by the spores rinsed from the fruit surface into the punctures.

In the 9294 punctures successful inoculation with *Penicillium expansum* was effected by this method in 95.5 per cent of the cases. In the computation of the probable error of the mean in each of the series, the number of successful inoculations was taken as n, the value of which can be determined from the data presented on the total number of punctures and the percentage of punctures decayed. The diameter of the decayed areas, the index found by Gregory and Horne (13) to be the best measure of the internal resistance, was taken as the criterion of susceptibility to the advance of decay.

The wide differences previously observed (4) in the rate of advance of *Penicillium expansum* in apples of uniform size, maturity, and history of development, with a definite amount of inoculum placed in punctures of uniform size, and held in storage of constant temperature and humidity, has been observed further in these studies. The difference in apples, and in punctures in the same apple, demand a high population in order to determine the true susceptibility. It is felt that a large population handled in the way outlined will give a truer picture of the mean susceptibility than will a low population inoculated and handled with more care. The usual formulae were used for computation of the probable error of the mean, probable error of the average of averages, and the probable error of the difference.

The following method of inoculation was employed to give a measure of the relative susceptibility of a series of apples to lenticel infection (*i.e.*, the mean number of susceptible lenticels per apple). Jonathan apples obtained from the Experiment Station orchard in Pullman and inoculated with *Penicillium expansum* by the method just outlined were held at room temperature until nearly decayed and then placed in cold storage. The

decayed tissue was stirred in a container, and enough of the juice decanted to leave a fairly firm paste. Some of this was coated thinly over each apple; the fruit was wrapped at once in a double thickness of oiled apple wrap, packed, and returned to storage. A fresh batch of this inoculum was made up each time a lot of fruit was inoculated. The effectiveness and accuracy of this method of determining the number of susceptible lenticels per apple has been discussed in an earlier publication (4). It should be noted that the results are in agreement with those previously obtained by dipping the fruit in a spore suspension before storage (4). As already reported, the difference is one of degree rather than type, and gives data qualitatively comparable to those obtained under commercial conditions. In the 1085 apples inoculated in this study only 3 (0.28 per cent) showed contamination and, in these cases, at one lenticel per apple. The contaminant was *Botrytis cinerea*.

EFFECT ON RESISTANCE OF THE APPLICATION OF ORCHARD FERTILIZERS

The literature concerning the effect of fertilizer application on the resistance of fruit to fungous decay is rather unsatisfactory. In some studies the fungi causing decay have not been determined, in others no distinction has been made between external and internal resistance of the fruit. Most of the investigations have dealt with weaker parasites than *Penicillium expansum*. This problem has been studied in England for a number of years in connection with investigations of the correlation of chemical composition of the fruit and resistance to various fungi. There has been a paucity of research on this subject in other countries.

There is rather general agreement that fruit from soils high in available nitrogen has a higher nitrogen content than fruit from check plots (1, 17, p. 284–289, 18, p. 292–300, 11, 24, 26), although there are some minor inconsistencies. Plagge (26) found that this increased nitrogen content was principally in the noncolloidal fraction, and that the decrease of this fraction in storage was more rapid in fruit from check plots than from those receiving nitrogen. The condition of high nitrogen content of the fruit has been shown to be correlated generally with high susceptibility to decay by such fungi as *Fusarium fructigenum* Fr., *Cytosporina ludibunda* Sacc., and *Diaporthe perniciosa* É. & É. March. (16, p. 135–139, 17, p. 284–289, 18, p. 292–300, 20), but some exceptions have been noted (19). Gourley and Hopkins (11) did not find a correlation between application of nitrogenous fertilizers to the soil and the amount of storage decay (unspecified cause) in the fruit produced. Carter (8) has given a possible explanation of these inconsistent results. He showed that *Pleospora herbarum* Pers., *Fusarium fructigenum*, *Polyopeus* sp., and *Alternaria* sp. varied with the pH in their response to the nitrogen content of the fruit. Apples of low acidity dis-

played decreasing susceptibility with an increased nitrogen content. The effect of the application of a given salt is conditioned further by the supply of it already available in the soil.

The published data on the effect of other fertilizers on the resistance of apples to fungous decay is less definite and consistent.

The effect of applications of nitrogen, potash, and phosphorus fertilizers, and their various combinations on the incidence of lenticel infection and the rate of advance of *Penicillium expansum* in the tissue, was investigated to some extent. Prime maturity Jonathan apples were obtained from the Experiment Station fertilizer plots in East Wenatchee. The fertilizers used were ammonium sulphate (N), superphosphate (P), and potassium chloride (K). Six pounds of superphosphate and 5 pounds of each of the other components were used annually per tree. The applicatons began in 1927, with the exception of NP and NK plots, which were started in 1928. The check plot had received no fertilizer since 1928. The fruit was picked, packed, and shipped at once to Pullman, where it was placed in storage.

The fruit of the 1930 series was punctured and inoculated on January 27, 1931, in the manner described and was held in storage at $-0.5°$ to $1°$ C. Measurements of the decayed areas on March 18, after an interval of 50 days, gave the results shown in table 1.

TABLE 1.—*Mean diameter of 50-day-old decayed areas in Jonathan apples from various fertilizer plots. 1930 series*

Fertilizer treatment	Total punctures	Percentage punctures decayed	Mean diameter (mm.)
N	222	96.85	29.637 ± .282
P	249	100.00	36.241 ± .349
K	222	99.55	29.880 ± .258
NPK	450	97.78	32.880 ± .176
Check	201	98.01	24.215 ± .303

The fruit of the 1933 test was picked at prime maturity on September 27–28 and was punctured and inoculated on December 17, 1933. The size of the lesions on January 16 (after 30 days) and February 15, 1934 (after 60 days), is given in table 2.

The results of the series of these 2 years justify no further conclusion than that the addition of fertilizers to the trees increased slightly the rate with which *Penicillium expansum* decayed fruit tissue. The application of any of these 3 salts alone or in combination decreased the internal resistance, but the degree of the effect was rather inconsistent throughout.

Organisms poorly adapted to the conditions of growth in the tissue of apples would be more strongly affected by alteration of chemical composition

TABLE 2.—*Mean diameter of decayed areas in Jonathan apples from various fertilizer plots 30 and 60 days after inoculation. 1933 series*

Fertilizer treatment	Total punctures	Examined after 30 days		Examined after 60 days	
		Percentage punctures decayed	Mean diameter (mm.)	Percentage punctures decayed	Mean diameter (mm.)
N	291	70.10	10.113 ± .183	96.91	27.608 ± .412
P	291	70.45	10.227 ± .144	98.28	25.734 ± .302
K	324	74.69	10.401 ± .133	97.84	29.598 ± .296
NP	297	77.44	9.783 ± .158	96.30	25.428 ± .410
NK	276	77.90	10.368 ± .170	96.74	28.554 ± .482
PK	297	73.06	8.952 ± .098	97.31	26.280 ± .258
NPK	285	77.54	10.515 ± .159	95.79	27.990 ± .383
Check	291	60.48	9.162 ± .157	89.35	24.096 ± .414

due to the application of fertilizers than would an active parasite such as *Penicillium expansum*. The omnivorousness of this fungus in nature, and its ability to grow readily on a wide variety of media (10, 29) lead one to expect only a slight effect on growth as a results of differences in the chemical composition of the fruit produced on trees in fertilizer and check plots. Certainly, the data show that modification of fertilizer practices alter internal resistance so slightly as to be unimportant in determining the incidence of blue-mold decay.

Another lot of the 1933 fruit, picked and stored in the same way, was coated with the tissue of decayed apples on December 17, 1933. On February 1, 1934, (46 days later) the fruit was examined and the number of lenticel infections by *Penicillium expansum* were recorded (Table 3).

TABLE 3.—*Number of lenticel infections in Jonathan apples from various fertilizer plots resulting from coating fruit with decayed tissue. 1933*

Fertilizer treatment	Number of apples	Percentage apples uninfected	Lenticel infections per apple	
			Mean	Maximum
N	40	2.50	7.375 ± .586	23
P	35	2.86	6.914 ± .631	21
K	42	9.52	5.714 ± .458	16
NP	36	0.0	12.667 ± .794	29
NK	32	6.25	7.188 ± .613	20
PK	40	2.50	8.425 ± .803	38
NPK	39	0.0	10.462 ± .832	28
Check	39	5.13	7.462 ± .476	24

No constant relationship was shown between the application of any of the 3 salts to the various plots and the resistance of the fruit to infection

at lenticels; these limited data suggest that fertilizer practices are unimportant in determining susceptibility to such infection.

EFFECT ON RESISTANCE OF MATURITY AT PICKING AND OF LENGTH OF STORAGE PERIOD

This problem has been studied in England for several years during investigation of the relation of chemical composition to decay resistance and to the fungous succession during prolonged storage. The major attention has been given to slow-growing fungi appearing late in the storage season, but it has been realized (21) that *Penicillium expansum* is able to infect at any time in storage. Later work (19) showed that, of the 7 fungi studied *P. expansum* was surpassed only by *Phomopsis coneglanensis* Trav. in ability to infect at lenticels.

Brooks and Cooley (7) found that longer time was required for the development of measurable rot when *Penicillium expansum* was inoculated into green fruit than when placed in ripe apples at 0° C. Overholser and Latimer (25) found, in California, that late pickings of several varieties of pears generally were more susceptible to blue-mold decay than earlier pickings. The amount of decay in Australian and New Zealand apples (including Jonathan and Delicious varieties) at the time of unloading in England was found in the years 1927–1930 to be directly related to the fruit maturity (12). The authors (4) previously found a slight progressive increase of the number of blue-mold lenticel infections in Jonathan, Delicious, and Winesap apples in early-, prime-, and late-maturity pickings; the number was greatly increased in extra-late maturity Jonathans.

The effect of maturity of fruit and the length of storage period on the incidence of lenticel infection and the rate of advance of decay in the tissue was investigated in 1933. Delicious apples were picked from 2 trees at early maturity (October 2, 1933); in order to accentuate the difference between early and prime pickings only the most immature fruit was selected for the former. The prime-maturity picking was made on October 11, 1933, from the same 2 trees. Winesap apples were picked from 2 trees at early maturity (October 2, 1933), and prime maturity (October 16, 1933). All lots were packed without being washed, and shipped at once to Pullman.

Part of the fruit of each box was punctured and inoculated by the method described, and a nonpunctured portion was coated with the tissue of apples decayed by *Penicillium expansum*. The first inoculation for early-maturity Delicious was made on October 6, and the late-maturity picking was first inoculated on October 14. For the Winesap variety the first dates were October 6 and October 19, respectively. Four subsequent inoculations were made at 60-day intervals from these dates, the last being made in June. The studies thus covered a period exceeding that of ordinary storage practice.

The punctured portion of each box was examined and the size of the lesions determined 30 days and 60 days after inoculation. The nonpunctured fruit, coated with decayed tissue, was examined 45 days after inoculation, and the number of lenticel infections per apple was recorded.

The data on the mean diameter of decayed areas in Delicious and Winesap apples of early- and prime-maturity pickings inoculated at different times in storage are presented in tables 4 and 5.

It is apparent that the rate of decay in the second 30-day period was greater than in the first 30 days; in the Delicious series the advance in the second period was about twice that of the first; in the Winesap it was about 1½ times that of the first period. This is in agreement with the conclusions of Brooks and Cooley (7) with the same organism.

TABLE 4.—*Mean diameter of decayed areas in early- and prime-mature Delicious apples inoculated at various periods in the storage life of the fruit*

Storage age of fruit (days)	Total punctures	Examined after 30 days		Examined after 60 days	
		Percentage punctures decayed	Mean diameter (mm.)	Percentage punctures decayed	Mean diameter (mm.)
Early-maturity picking					
0	261	96.17	13.140 ± .152	100.00	35.604 ± .242
60	240	78.75	11.520 ± .131	97.50	33.680 ± .353
120	249	26.10	10.754 ± .266	98.31[a]	29.268 ± .392
180	261	65.13	10.953 ± .143	100.00	38.430 ± .271
240	129	80.62	16.590 ± .362	100.00	45.384 ± .499
Total or average	1140	68.33	12.591 ± .102	99.11[a]	36.473 ± .162
Prime-maturity picking					
0	213	99.53	14.717 ± .236	99.53	41.310 ± .386
60	228	81.58	10.746 ± .150	99.56	34.224 ± .327
120	228	32.89	9.120 ± .177	99.56	33.304 ± .218
180	222	86.76[b]	10.464 ± .151	100.00[b]	39.168 ± .242
240	261	97.67[c]	21.202 ± .188	100.00[d]	51.475 ± .120
Total or average	1152	79.84[e]	13.250 ± .082	99.73[f]	39.896 ± .123

[a] Exclusive of 4 apples infected by *Botrytis cinerea.*

[b] Exclusive of 1 apple infected by *B. cinerea.*

[c] Exclusive of 3 punctures in 3 apples infected by *B. cinerea.*

[d] Exclusive of 7 apples infected by *B. cinerea.*

[e] Exclusive of 6 punctures infected by *B. cinerea.*

[f] Exclusive of 8 apples infected by *B. cinerea.*

The prime-maturity fruit consistently showed somewhat larger decayed areas than early-maturity pickings. The difference in the Delicious pickings

was 0.659 ± .130 mm. after 30 days, and 3.423 ± .202 mm. after 60 days. With the Winesap variety the difference was 1.201 ± .110 mm. after 30 days, and 3.518 ± .200 mm. after 60 days. Although these figures are statistically significant, the differences are rather small. In these series the percentage of punctures decayed showed that the prime-maturity fruit was slightly more susceptible to infection than that harvested earlier. It is felt that maturity at the time of picking is of small importance in determining internal resistance to the advance of *Penicillium expansum*, even though fruit picked early is slightly more resistant than that harvested at prime maturity.

TABLE 5.—*Mean diameter of decayed areas in early- and prime-mature Winesap apples inoculated at various periods in the storage life of the fruit*

Storage age of fruit (days)	Total punctures	Examined after 30 days		Examined after 60 days	
		Percentage punctures decayed	Mean diameter (mm.)	Percentage punctures decayed	Mean diameter (mm.)
Early-maturity picking					
0	360	58.33	8.736 ± .152	99.72	19.386 ± .252
60	348	25.29	10.293 ± .230	67.53	18.128 ± .402
120	342	28.36	8.784 ± .182	90.56[a]	21.696 ± .347
180	339	32.45	9.543 ± .114	99.12	31.500 ± .237
240	312	72.76	13.344 ± .182	99.68	38.958 ± .202
Total or average	1701	43.03	10.140 ± .079	90.99[a]	25.934 ± .133
Prime-maturity picking					
0	327	78.59	11.883 ± .151	100.00	27.991 ± .357
60	309	60.19	9.969 ± .177	90.29	25.806 ± .381
120	318	29.56	8.340 ± .178	86.48	23.682 ± .319
180	366	33.61	9.561 ± .122	96.16[b]	28.764 ± .299
240	285	88.73[b]	16.952 ± .200	98.92[c]	41.018 ± .274
Total or average	1605	56.86[b]	11.341 ± .075	94.36[d]	29.452 ± .147

[a] Exclusive of 1 apple infected by *Botrytis cinerea.*

[b] Exclusive of 1 puncture infected by *B. cinerea.*

[c] Exclusive of 7 punctures infected by *B. cinerea.*

[d] Exclusive of 8 punctures infected by *B. cinerea.*

The effect of length of the storage period on the internal resistance of Delicious and Winesap apples to the advance of *Penicillium expansum* was variable in these investigations. Only small differences were noted during the first 120 days, but marked increases in the rate of advance occurred after 180 days, particularly with prime-maturity fruit. The ability

of this organism to grow readily on a wide range of substrata (10, 29) probably explains the fact that no consistent effect on growth rate occurred until late in the season. These results are not in disagreement with the findings of English investigators with less omnivorous and virulent pathogens. In general the internal resistance of apples to *P. expansum* may be said to be unimportant in determining the incidence of blue-mold decay in commercially stored fruit. As a single visible decay spot makes the apple a cull, a decrease in the rate of advance of decay is of commercial significance only in the early stages of invasion; the mere extension of the time required to produce complete decay is unimportant. The prolonged storage periods employed give ample time for the visible expression of infections of the wounds resulting from packing operations and of infections early in the season. Late-season lenticel infections and infections at wounds incurred in market handling become apparent rather rapidly, due to decreased internal resistance.

The effect of maturity of fruit and the length of the storage period on the incidence of lenticel infection in fruit coated with decayed tissue is shown in tables 6 and 7.

The prime-maturity picking showed more lenticel infections per apple than the early-maturity fruit in both the Delicious and Winesap varieties.

TABLE 6.—*Number of lenticel infections in early- and prime-mature Delicious apples coated with decayed tissue at various periods in the storage life of the fruit*

Storage age of fruit (days)	Number of apples	Percentage apples uninfected	Lenticel infections per apple	
			Mean	Maximum
Early-maturity picking				
0	36[a]	0	7.000 ± .533	20
60	36	5.56	9.083 ± .757	34
120	37	2.70	17.459 ± 1.549	50
180	38	2.63	7.632 ± .625	26
240	31	6.45	11.742 ± .931	35
Total or average	178[a]	3.47	10.583 ± .425	33
Prime-maturity picking				
0	26	0	30.654 ± 2.931	106
60	30	3.33	14.200 ± 1.060	37
120	32	0	13.375 ± 1.030	40
180	29	3.44	11.448 ± 1.140	33
240	33	0	18.182 ± 1.177	44
Total or average	150	1.35	17.572 ± .734	52

[a] Including 3 apples each with one infection by *Botrytis cinerea* in addition to those by *Penicillium expansum*.

TABLE 7.—*Number of lenticel infections in early- and prime-mature Winesap apples coated with decayed tissue at various periods in the storage life of the fruit*

Storage age of fruit (days)	Number of apples	Percentage apples uninfected	Lenticel infections per apple	
			Mean	Maximum
Early-maturity picking				
0	50	24.00	2.740 ± .237	11
60	43	55.81	1.293 ± .079	4
120	53	43.00	1.849 ± .174	8
180	48	50.00	1.438 ± .103	5
240	43	4.65	6.721 ± .576	22
Total or average	237	35.57	2.808 ± .132	10
Prime-maturity picking				
0	46	2.17	6.348 ± .712	30
60	38	23.68	2.974 ± .339	16
120	44	29.55	3.523 ± .421	17
180	50	16.00	4.240 ± .366	17
240	39	0	6.615 ± .569	19
Total or average	217	14.28	4.740 ± .224	19.8

The difference in the case of the former was 6.989 ± 0.848 and in the latter was 1.932 ± 0.259; both of these figures are statistically significant. These differences are rather large in comparison with the average number of lenticel infections involved, being nearly half of the prime-maturity average in each case. The trend is the same as that obtained in 1931 (4) with Jonathan, Delicious, and Winesap apples inoculated with a spore suspension. From the standpoints of the maximum number of lenticel infections, the trend of increased susceptibility of the lot with increased picking maturity is also apparent. It is, therefore, concluded that the susceptibility of these varieties to lenticel infection (*i.e.*, the number of susceptible lenticels per apple) is increased materially by delayed harvesting.

There was shown in this study no consistent relation of the length of storage period and the susceptibility of the fruit to lenticel infections. Thus the number of infectible lenticels per apple did not change materially during storage. This is not surprising, since cutinization, the process involved in the development of resistant lenticels (9), does not proceed rapidly at low temperatures, and since the epidermal and subepidermal cells may die early in the storage season (2). It was found previously (4) that holding the fresh fruit at orchard temperature and humidity for 6 or 12 days or in dry air at 30° C. for 10 days tended to decrease the number of infectible lenticels per apple. Horne (19), working with *Penicillium expansum* and 6 other fungi, stated that "the results failed to show that age had any appreciable effect in modifying the external resistance."

VARIETAL SUSCEPTIBILITY

The varietal susceptibility of apples to infection at lenticels and to the radial advance of *Penicillium expansum* has received little attention.

Machacek (23) reported that the apple variety Russet was apparently "immune to outside infection." The destructive pinhole rot of pears on the Pacific Coast, a result of blue-mold lenticel infection, is much more prevalent in Winter Nelis than in any other variety (14, 27). Columbia, Doyenné d'Alencon, and Seckel pears have been reported (25) to be relatively immune from blue-mold decay, "apparently on account of the character of the tissues and epidermis." In tests with over 19,000 apples of the 1931 crop handled in various ways, the writers (4) found the decreasing order of susceptibility to infection at lenticels to be: Jonathan, Delicious, Winesap. Horne and Eweis (19) found that Worcester Pearmain apples were more susceptible than Bramley's Seedling to lenticel infection by *Penicillium expansum* and 4 other fungi.

Some work has been conducted to determine the susceptibility of different varieties of apples and pears to the radial advance of *Penicillium expansum* in the tissues. Machacek (23) found that blue-mold decay spread more rapidly in McIntosh than in Fameuse apples. Unpublished data[5] of R. W. Welsh on the rate of advance of *P. expansum* in a limited number of apples of 12 varieties held at about 22° C. showed the decreasing order of resistance to be: York, Stayman Winesap, Winesap, Rome Beauty, Jonathan, Baldwin, Ben Davis, McIntosh, Wealthy, Delicious, Golden Delicious, and Grimes. The fruit was used at only one stage of maturity, and was inoculated on only one date, November 7, 1931.

The data presented in tables 4 and 5 consistently show that the rate of decay in the Delicious variety was more rapid than in the Winesap. In the early-maturity pickings the difference in mean diameter of the decayed area after 60 days was $10.539 \pm .210$ mm., and in the prime-maturity series it was $10.444 \pm .192$ mm. The same trend was shown by the percentage of punctures infected in each of these lots. The figures on the mean diameter of decayed areas were computed with the number of infected punctures taken as n and are, therefore, independent of the percentage of punctures that developed decay.

The data in tables 6 and 7 show that the Delicious was also consistently more susceptible to infection at lenticels than was the Winesap variety. The difference in the number of lenticel infections per apple in the early-maturity series was $7.775 \pm .445$, and in the prime-maturity fruit was $12.832 \pm .768$. The same relationship was displayed in 2 other ways; the Delicious showed a much lower percentage of noninfected fruit and higher maximum number of infections per apple than the Winesap.

[5] Detailed data supplied by Dr. C. R. Orton, West Virginia Agricultural Experiment Station. Brief abstract in West Virginia Agr. Expt. Sta. Bull. 254: 52. 1932.

Varieties of apples differ greatly in the length of time they may be stored before they show senility and physiological breakdown. It has been shown that the susceptibility to lenticel infection by *Penicillium expansum* and the rate of advance of decay caused by it increase with delayed harvest and prolonged storage. A highly susceptible variety may be more resistant at the time of picking than a resistant one taken at the end of its storage life. It is apparent that any general study of varietal susceptibility to this organism must include tests on several stages of picking maturity made at a number of periods in the storage life. With varieties more dissimilar than those here employed, it is expected that a greater effect of time on internal resistance and the number of infectible lenticels would be shown. These points should be considered in studies of varietal susceptibility of apples to other decay fungi.

DISCUSSION AND CONCLUSIONS

In the limited data presented, no appreciable effect of the application of orchard fertilizers was shown on the incidence of susceptible lenticels, but decayed areas in apples from fertilizer plots consistently were somewhat larger than in fruit from check plots. Inasmuch as incidence rather than increment of rot is a criterion of condition in commercial handling, and as the number of lenticels and punctures infected is not greatly influenced by fertilizer applications, orchard fertilization is probably unimportant in determining the occurrence of blue-mold decay in the stored fruit. This is thought to be due to the ability of *Penicillium expansum* to grow readily on a wide range of substrata; less omnivorous fungi might be expected to show a greater sensitivity.

The percentage of punctures successfully infected, and the rate of spread of blue-mold in Delicious and Winesap apples picked at prime maturity was only slightly greater than in fruit picked at an earlier date. However, in these and earlier (4) investigations there was a significant increase in the mean number of infectible lenticels per apple as a result of delayed harvest. The same trend was shown by the percentages of apples without susceptible lenticels and by the maximum number per apple in each of the series. It is not known why the number of open lenticels per apple should increase with maturation on the tree, but decrease with holding of the picked fruit at orchard temperatures for a few days (4). The number of open lenticels per apple was shown previously to be very high in an extra-late maturity picking of Jonathan. It seems fairly clear that delayed harvesting is an important factor in determining the amount of blue-mold decay in certain lots of stored fruit. The increase in the number of open lenticels with the attendant increased chance of the apple becoming infected is of more commercial consequence than the increased rate of spread of decay in an apple that is already a cull.

The internal resistance of Delicious and Winesap apples varied little during storage until April (180 days), after which the rate of advance of blue-mold decay materially increased. The number of susceptible lenticels per apple sometimes showed this same increase, but to a lesser degree. It is thought that the slight effect of this factor was due to the good keeping quality of the varieties used; with early varieties, or varieties of short storage life, the effect would be greater after such periods of storage.

The English investigators have considered that a localized senescence in the region of the lenticel preceded infection of it by fungi, and that the advance of the weaker pathogens was then conditioned by the general senescence (*i.e.*, lowered internal resistance) of the tissue. This theory is best presented by Kidd (21). It is not yet possible to say definitely whether the senescent tissue-lenticel infection theory can be applied to *Penicillium expansum*. The evidence for such a relation is: (a) the sudden advance of incipient lenticel infections after removal of fruit from cold storage (4); (b) the increase in the number of susceptible lenticels per apple and the decrease of internal resistance with delayed harvesting or prolonged storage. On the other hand the omnivorousness of *P. expansum* and the lack of marked internal resistance to it in apples, the correlation of susceptibility and the noncutinized condition of lenticular cells (4, 9), the fact that reduction of the number of susceptible lenticels by exposure to warm dry air (4, 9) would be impossible if senescence were the factor involved, and the unimportance of the "vigor of development" of the fungus in the process of infection (4)—all indicate that localized senescence is unimportant in determining lenticular resistance to *P. expansum*.

Under the conditions of this and previous (4) tests the resistance, expressed either in terms of the rate of advance of decay or as the number of susceptible lenticels per apple, was greater in the Winesap than in the Delicious variety. The internal resistance was indicated by the mean diameter of lesions and by the number of punctures infected; the resistance to lenticel infection was expressed as the mean and maximum number per apple, and by the percentage of apples without susceptible lenticels.

The resistance of the lenticels to infection by *Penicillium expansum* is associated with cutinization of the cells forming the lenticel basin (9). It seems probable that the susceptibility of an apple to lenticel infection is a function of the number of open lenticels in it. Whether the internal resistance is due to the physical structure, the chemical composition, or both, is unknown; the English work with other fungi suggests that the second may be the main factor.

The evidence at hand indicates that the number of infectible lenticels per apple may be increased by delayed harvesting, by holding fruit in cold storage and subsequently washing it in hot solutions, by severely bruising the apples, and by prolonged storage.

Jonathan, Delicious, and Winesap, in the order named, show decreasing susceptibility to lenticel infection by *Penicillium expansum* and, in the case of the last 2, to its radial advance in the tissue. It is perhaps significant that this is also the increasing order of the length of storage life and of commercial importance of the varieties in Washington.

The practical application of these findings to the Washington apple industry is rather obvious. The application of orchard fertilizers, the varieties grown, and the length of time fruit is held in storage may well continue to be determined without consideration of their relation to incidence of blue-mold decay. However, the practice of leaving fruit on the tree past prime maturity should be discontinued unless decay has been found to be unimportant in the grower-lot in question. A detailed consideration of the methods for the prevention of blue-mold decay under Washington conditions has been presented in another place (5).

SUMMARY

The rate of advance of blue-mold decay in Delicious and Winesap apples under Washington conditions was found to be slightly greater in fruit from trees in fertilizer plots than from trees in check plots, and greater in fruit picked at prime maturity than at early maturity. The length of the storage period did not affect the rate of decay until after 180 days, when it materially increased.

The number of lenticel infections by *Penicillium expansum* per apple was greater in fruit picked at prime maturity than at early maturity. The incidence of such infections in apples was unaffected by the application of orchard fertilizers and by the length of the storage period up to 180 days, but after this period a slight increase sometimes was shown.

The Winesap variety was more resistant to lenticel infection by *Penicillium expansum* and to the radial advance of this fungus in the tissue than was the Delicious.

Fruit should be picked when mature in order to prevent decrease of its resistance to blue-mold decay. The other cultural and handling practices investigated should be determined by factors other than occurrence of this storage rot.

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LITERATURE CITED

1. ALDRICH, W. W. Effect of fall applications of sodium nitrate upon the color, keeping quality and nitrogen content of apples. Maryland Agr. Expt. Sta. Bull. 326. 1931.
2. ARTHUR, J. M. Red pigment production in apples by means of artificial light sources. Contrib. Boyce Thompson Inst. 4: 1–18. 1932.

3. BAKER, K. F., and F. D. HEALD. The importance of lenticel infection of apples by *Penicillium expansum*. Washington Agr. Expt. Sta. Bull. 264. 1932.
4. ————, and ————. An investigation of factors affecting the incidence of lenticel infection of apples by *Penicillium expansum*. Washington Agr. Expt. Sta. Bull. 298. 1934.
5. ————, and ————. Investigations on methods of control of the blue-mold decay of apples. Washington Agr. Expt. Sta. Bull. 304. 1934.
6. BARNUM, C. C. Stem end rot of apples. Science (n.s.) **55**: 707–708. 1922.
7. BROOKS, C., and J. S. COOLEY. Temperature relations of apple-rot fungi. Jour. Agr. [U. S.] Res. **8**: 139–164. 1917.
8. CARTER, F. M. Investigations of factors affecting advance of certain 'apple spot' fungi within the host tissue. Ann. Bot. [London] **48**: 363–394. 1934.
9. CLEMENTS, H. F. Morphology and physiology of the pome lenticels of *Pyrus malus*. Bot. Gaz. **97**: 101–117. 1935.
10. FRASER, L. An investigation of the sooty-moulds of New South Wales. II. An examination of the cultural behavior of certain sooty mould fungi. Linn. Soc. N. S. Wales Proc. **59**: 123–142. 1934.
11. GOURLEY, J. H., and E. F. HOPKINS. Nitrate fertilization and keeping quality of apple fruits. Chemical, physiological, and storage studies. Ohio Agr. Expt. Sta. Bull. 479. 1931.
12. GT. BRIT. EMPIRE MARKETING BD. Australian and New Zealand fruit shipments. Report of an investigation by the Economic section of the Empire Marketing Board into the deterioration in transit of imported Australian and New Zealand fruit, 1927–30. [Gt. Brit.] Empire Marketing Bd. Pub. 46. 1931.
13. GREGORY, F. G., and A. S. HORNE. A quantitative study of the course of fungal invasion of the apple fruit and its bearing on the nature of disease resistance. Part I. A statistical method of studying fungal invasion. Roy Soc. [London] Proc. Ser. B, **102**: 427–443. 1928.
14. HARTMAN, H. The handling of pears on eastern markets. Ice and Refrig. **82**: 373–374. 1932.
15. HEALD, F. D., J. R. NELLER, F. L. OVERLEY. Arsenical spray residue and its removal from apples and pears. Washington Agr. Expt. Sta. Bull. 226. 1928.
16. HORNE, A. A. Biological work [on fruit]. [Gt. Brit.] Dept. Sci. and Indus. Res. Food Inves. Bd. Rept. **1929**: 125–144. 1930.
17. ————. Biological work on fruit. [Gt. Brit.] Dept. Sci. and Indus. Res. Food Inves. Bd. Rept. **1931**: 272–289. 1932.
18. ————. Biological work on fruit. [Gt. Brit.] Dept. Sci. and Indus. Res. Food Inves. Bd. Rept. **1932**: 279–300. 1933.
19. ————. Biological work on fruit. [Gt. Brit.] Dept. Sci. and Indus. Res. Food Inves. Bd. Rept. **1933**: 228–245. 1934.
20. HORNE, A. S., and F. G. GREGORY. A quantitative study of the course of fungal invasion of the apple fruit and its bearing on the nature of disease resistance. Part II. The application of the statistical method to certain specific problems. Roy. Soc. [London] Proc. Ser. B. **102**: 444–466. 1928.
21. KIDD, M. N. The mortality curve in a population of apples. [Gt. Brit.] Dept. Sci. and Indus. Res. Food Inves. Bd. Rept. **1924**: 43–46. 1925.
22. ————, and A. BEAUMONT. An experimental study of the fungal invasion of apples in storage with particular reference to invasion through the lenticels. Ann. Appl. Biol. **12**: 14–33. 1925.
23. MACHACEK, J. E. Studies on the association of certain phytopathogens. Macdonald Col. McGill Univ., Tech. Bull. **7**. 1928.

24. ONSLOW, M., F. KIDD, and C. WEST. Biochemical study of senscence in apples. [Gt. Brit.] Dept. Sci. and Indus. Res. Food Inves. Bd. Rept. 1931: 52–77. 1932.
25. OVERHOLSER, E. L., and L. P. LATIMER. The cold storage of pears. California Agr. Expt. Sta. Bull. 377. 1924..
26. PLAGGE, H. H. The storage behavior of apples as influenced by nitrogen fertilization and storage temperature. Iowa State Col. Jour. Sci. 9: 95–114. 1934.
27. ROSE, D. H., C. BROOKS, D. F. FISHER, and C. O. BRATLEY. Market diseases of fruits and vegetables: apples, pears, quinces. U. S. Dept. Agr. Misc. Publ. 168. 1933.
28. STEVENS, N. E., and N. W. NANCE. Efficiency of oiled wraps in the commercial control of apple scald. Phytopath. 22: 603–607. 1932.
29. THOM, C. Cultural studies of species of Penicillium. U. S. Dept. Agr. Bur. Anim. Indus. Bull. 118. 1910.
30. U. S. DEPT. AGR., BUR. AGR. ECON. Statistics of importance crops, by states. Apples. U. S. Dept. Agr., Crops and Market 3: 401. 1926; 4: 465. 1927; 5: 455. 1928; 6: 472. 1929; 8: 513. 1931.
31. ZSCHOKKE, A. Ueber den Bau der Haut und die Ursachen der verschiedenen Haltbarkeit unserer Kernobstfrüchte. Landw. Jahrb. Schweiz. 11: 153–197. 1897.

LIBERATION OF NEUTRALIZED VIRUS AND ANTIBODY FROM ANTISERUM–VIRUS PRECIPITATES[1]

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It has been known for 10 years that, when normal rabbit blood serum is mixed with plant juice containing the virus of tobacco mosaic, the serum causes a reduction in the number of infections obtainable with the virus juice. If the serum of an animal that has been immunized with the virus is used in this way, the reduction in infectivity is much more marked (8, 10, 11, 12). The writer pointed out in 1934 (4) that both types of serum decrease the infectivity of the virus through a nonspecific inhibitory effect, and that immune serum, in contrast to normal serum, also has a specific neutralizing effect on the virus. A further study has been made on the nature of the neutralizing action of serum on virus, and the present paper reports the results of this study. Since the nonspecific inhibitory effect of normal serum constituents seriously interferes with the study of specific neutralization, it was found desirable to precede the work on neutralization by attempts to purify the serum so as to eliminate the nonspecific inhibitory factor as far as possible. The first section of this paper is, accordingly, devoted to experiments in antibody purification.

PURIFICATION OF VIRUS-IMMUNE SERUM

Numerous techniques have been used, up to the present, in the purification and concentration of antibodies. The techniques include desiccation, moderate heating, fractionation with ammonium sulphate, dialysis and electro-dialysis, precipitation by dilution, adsorption and elution, and treatment with copper chloride. An adequate presentation of this subject and introduction to the literature concerned has been given by Hartley (7).

The method finally adopted in the present case consisted of a moderate heating followed by ammonium sulphate precipitation and dialysis. Virus-immune rabbit serum was obtained in the customary manner and absorbed with healthy-tobacco juice to remove the precipitins for healthy-tobacco protein (5). The absorbed serum was heated at 57° C. for 2 hours. This process converts some of the water-soluble globulin into water-insoluble globulin, without affecting the antibody content. Protein was then precipitated by adding saturated ammonium sulphate solution until the ammonium sulphate

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concentration reached 30 per cent saturation. This precipitated protein fraction (euglobulin), which showed very little antibody activity, was filtered off and discarded. The filtrate was next precipitated by adding enough saturated ammonium sulphate to bring the ammonium sulphate concentration up to 43 per cent saturation. The resultant protein precipitate (pseudoglobulin) contained nearly all the antibodies of the serum, but only a small proportion of the total protein of the serum. The filtrate from this precipitation (albumin) contained much protein but was nearly or entirely free from antibodies. The pseudoglobulin precipitate was suspended in water or saline solution and dialyzed against water. During dialysis there was a heavy precipitation of water-insoluble globulin. This precipitation did not remove appreciable amounts of antibody. After dialysis the precipitate was removed by centrifuging, and to the supernatant fluid was added enough concentrated NaCl solution to bring the salt concentration to .85 per cent. Such purified serum was colorless, water-clear, and highly active, serologically.

The various protein fractions of normal serum were tested in a number of experiments to determine which fractions contained the substance or substances responsible for the nonspecific inhibitory property of serum. The results of the tests showed that this property is associated with the proteins, the dialyzable salt fraction being noninhibitory. Of the proteins, the albumin and euglobulin were each somewhat inhibitory to virus infection but less so than the pseudoglobulin. However, the removal of both the albumin and euglobulin fractions reduced the nonspecific inhibitory action of serum to a very considerable extent without affecting the antibody content.

As this method of purification of immune serum greatly reduces the protein content of the serum, it was thought that the precipitin titer of the purified serum might be unduly low, since it was possible that the nonantibody protein of the serum might contribute to the bulk of precipitate produced in the precipitin reaction. The nonantibody protein content of purified serum was, therefore, increased by adding various amounts of nontreated normal serum. No increase in precipitin titer resulted from this addition, indicating that in the purified serum there is sufficient protein to permit a maximum precipitin reaction.

Having developed a method for the purification of immune serum that removed a large proportion of the materials responsible for the host-inhibitory effect of the serum without removing the antibodies, it was feasible to proceed to an investigation of the nature of the neutralization reaction. The work was developed along two lines, namely, a study of the recovery of virus after neutralization with immune serum and a study of the recovery of antibody from serum-virus mixtures that contained no serologically demonstrable free antibody.

LIBERATION OF NEUTRALIZED VIRUS

Numerous methods for the purpose of recovering virus from serum-virus mixtures have been attempted in the past, but with relatively little success. Vaccinia virus is an exception. If this virus is treated with an adequate amount of serum from a recovered animal, the virus is neutralized so that a dose of the mixture administered to a normal animal fails to cause disease. If to a similar dose that would fail to cause infection are added several parts of saline diluent and the whole diluted mixture is inoculated into an animal, disease results (1). In this case the noninfective virus-antibody combination apparently has been dissociated simply by dilution. Reactivation by dilution also has been described for the viruses of fowl-plague, herpes, and poliomyelitis (2). Vaccinia virus and virus III of rabbits have both been recovered from neutral virus-serum mixtures by adsorption on kaolin or euglobulin (1). Techniques of washing, heating, and change in pH have all been utilized in attempts to separate virus and antibody. Weiss (14) reported the recovery of bacteriophage from a neutral bacteriophage-antiserum mixture through a digestion of the mixture with trypsin, but this was not confirmed by Muckenfuss (9). No other record of liberation of neutralized virus by enzyme action has come to hand.

In preliminary experiments, a number of techniques were used in attempts to recover tobacco-mosaic virus after neutralization. These techniques included dilution in saline solution or water, heating nearly to the thermal inactivation point of the virus, titrating with acid or alkali nearly to the extinction point of the virus, and treating with various chemicals, *e.g.*, .125 per cent $AgNO_3$, .0625 per cent $KMnO_4$, and .25 per cent chloramine-T. Virus antigen was not recovered when either serum-virus mixtures or the washed precipitate from such mixtures were treated in any of these manners.

It was thought that the immunization of a rabbit with serum immune from tobacco-mosaic virus might induce in the rabbit antibodies antagonistic to the action of the immune serum with which it was injected, *i.e.*, "anti-antibodies." This procedure was attempted. Rabbits were inoculated with 36 cc. of whole virus-immune serum, given in 6 biweekly intraperitoneal inoculations. The serum from the rabbits was mixed in various dosages with ordinary anti-mosaic serum and the mixtures tested for precipitating power against virus juice. It was found that the "anti-antiserum" had no demonstrable effect in inhibiting the action of the mosaic-immune serum. On the contrary, it behaved exactly like an ordinary anti-mosaic serum of rather low titer. The experiment shows that when mosaic-immune rabbit serum is inoculated into rabbits, it does not behave like a foreign body in inducing formation of antagonistic antibodies.

In addition to the experiments mentioned above, attempts were made to liberate neutralized antibody or virus by digestion of the serum-virus mix-

tures with pepsin. This procedure proved successful. The pepsin, under appropriate conditions, digested the antibody leaving the virus free and capable of acting both as a precipitinogen and as an infectious agent. Accordingly, the experiments with pepsin will be considered in some detail.

For recovery of virus precipitinogen, the following procedure was employed. Absorbed anti-virus serum was titrated against virus juice until neutralization was as complete as possible. In such neutralized mixtures virtually all of the virus and antibody were thrown down in the precipitate, leaving a supernatant in which precipitin tests showed the presence of no free virus or antibody. The more delicate infectivity test usually revealed some free virus in the supernatant fluids after neutralization. Even if a large excess of serum is added to virus, a few infections may be obtained from the mixtures, although relatively little serum is required to neutralize most of the virus. In this respect the neutralization of tobacco-mosaic virus by its specific antibody appears to resemble the neutralization of toxin by antitoxin. In the present study, mixtures of serum and virus were considered neutral when precipitin tests revealed no excess antibody or virus in the supernatant fluids of the mixtures. Infection tests of such mixtures showed a loss of most of the original infectivity of the virus used.

The mixtures or the washed precipitates from the mixtures after proper incubation were acidified and treated with crystalline pepsin, which was available through the courtesy of Dr. Roger M. Herriott. After incubation, the serum-virus-pepsin mixtures were titrated to pH 7 and centrifuged. The supernatant fluids then were found to contain large quantities of virus. This was detected either by precipitin tests or by infectivity tests. The following experiment is representative of several in which the precipitin method was used to detect liberated virus.

Absorbed anti-mosaic serum was purified according to the technique described in the previous section. To 1-cc. samples of the serum were added amounts of a concentrated virus juice varying from 2 cc. to .05 cc., with saline solution to adjust all the mixtures to the same volume. The mixtures were shaken and incubated for 2 hours at 37° C. and then overnight on ice. The following morning the amount of precipitate in each tube was noted. All of the tubes were centrifuged and the precipitates discarded. The supernatant fluids were each tested against undiluted absorbed anti-mosaic serum and against tobacco-mosaic juice at dilutions of 1:1 and 1:10. It was found that mixtures in which 1 cc. of serum had been added to more than .25 cc. of virus juice still contained excess virus, as shown by their reactions with the immune serum, while mixtures in which less than .25 cc. of virus juice had been added contained an excess of serum, as shown by precipitin tests. Accordingly, the mixture of 1 cc. serum with .25 cc. virus juice contained the optimum neutralizing proportions of serum and virus. A large amount of

serum-virus mixture was then made by adding 4 parts serum to 1 part virus juice. The mixture was incubated in the usual manner. The following morning a sample was centrifuged, tested, and found to contain an excess of neither serum nor virus. The mixture was then titrated to pH 3 and divided into 7 pairs of 1.5-cc. samples. To each sample was added concentrated pepsin and enough saline solution to adjust the volume to 3.5 cc. Each pair received a different amount of pepsin. One member of each pair was titrated back to pH 7.0 at once. This destroys the pepsin before it has time to act. The other member was titrated back to pH 7.0 after 5 hours' incubation at 37° C. The two members of each pair were treated identically throughout, except for the time when alkali was added. The 14 samples after incubation were dialyzed against saline solution for 16 hours and centrifuged. The supernatant fluids were then tested for precipitin reactions against tobacco-mosaic-immune serum and tobacco-mosaic-virus juice. The results of the tests are given in table 1.

TABLE 1.—*Recovery of neutralized virus by pepsin digestion, as shown by precipitin tests*

Sample	Pepsin[a]	Incubation[b]	Precipitin test with					Residual[c] precipitate
			Virus-immune serum at:				Virus juice at:	
			1:1	1:2	1:4	1:8	1:10	
1	0.01	5 hrs.	t[d]	t	t	t	0	++
2	0.03	"	++	++	++	++	0	+
3	0.1	"	+++	+++	+++	+++	0	0
4	0.3	"	+++	+++	+++	+++	0	0
5	1.0	"	+++	+++	+++	+++	0	0
6	3.0	"	++	++	++	++	0	0
7	8.0	"	+	+	+	+	0	0
8	0.01	None	0	0	0	0	0	+++
9	0.03	"	0	0	0	0	0	+++
10	0.1	"	0	0	0	0	0	+++
11	0.3	"	0	0	0	0	0	+++
12	1.0	"	0	0	0	0	0	+++
13	3.0	"	0	0	0	0	0	+++
14	8.0	"	0	0	0	0	0	+++

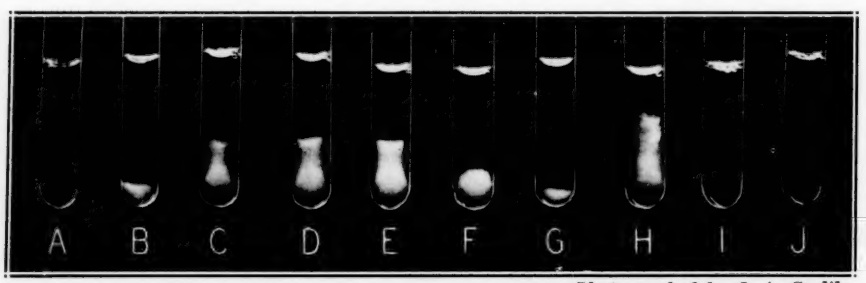
[a] Final concentration of crystalline pepsin in mg. of pepsin protein nitrogen per cc.

[b] Period elapsing between addition of pepsin to acid mixture and titration to pH 7.0

[c] Undigested precipitate from original precipitin reaction after pepsin treatment and titration to pH 7.0.

[d] +++ = strong reaction.
++ = moderate reaction.
+ = reaction safely positive but not strong.
t = trace of reaction.

Consideration of the table brings out the following facts. Each of the mixtures on which pepsin was allowed to act for 5 hours subsequently yielded a supernatant fluid that contained virus precipitinogen, as shown by its reaction with mosaic-immune serum. In other words, virus that had been bound by the anti-virus antibodies was released by the digestion of the antibodies by pepsin. With .01 mg. protein nitrogen (P. N.) pepsin per cc., the release was small, and there still remained undigested precipitate; in the mixture receiving .03 mg. P. N. pepsin there was greater release of virus and less undigested precipitate. In the tubes receiving .1, .3, or 1.0 mg. P. N. pepsin, all of the precipitate was digested and a maximum amount of virus was released. In the tubes that received 3.0 and 8.0 mg. P. N. pepsin, all of the precipitate was digested, and at this high concentration of pepsin not only were the antibodies destroyed but part of the virus as well, as is shown by the decreasing precipitin reaction. In the control tubes receiving various amounts of pepsin, but in which the pepsin was not allowed time to act, there was no indication of digestion of the original precipitate, nor was any liberated virus demonstrable. Figure 1 is from a photograph of some of the tubes



Photographed by J. A. Carlile

FIG. 1. Recovery of neutralized virus by pepsin digestion, as determined by the precipitin method.

from this experiment. Tubes A–G show the precipitin reactions of the released virus in tubes 1–7 of table 1, respectively. Tube H shows the amount of precipitate that would have been obtained had all the virus been recovered. Tubes I and J are representative of the precipitin tests of mixtures that contained pepsin that was not allowed time to act. It will be seen that the yield of recovered virus is relatively high in tubes C, D, and E when these are compared with the yield in tube H.

The pepsin dosages for maximum virus recovery are rather high (.1 to 1.0 mg. P. N. pepsin per cc.) as compared with the amounts of pepsin usually required for protein digestion. This is in accordance with the known fact that antibodies in general are more highly resistant to enzymes (and other physical and chemical agencies) than are the other serum proteins. The antibodies for tobacco-mosaic virus appear to be more stable than the antibodies for certain other plant viruses.

It is concluded from these experiments that the digestion of a neutral mixture of tobacco-mosaic virus and its specific serum with pepsin liberates large amounts of virus, as shown by precipitin tests. It was felt that supplementary evidence of the liberation of virus should be obtained from experiments in which pepsin digestion of serum-virus mixtures was followed by infection tests. Such experiments were accordingly performed.

The serum-virus mixtures from experiments such as that described above were tested for infectivity to determine whether the recovery of virus antigen was correlated with a recovery of virus infectivity. It was soon learned that an adequate demonstration of the recovery of virus infectivity after pepsin digestion of a neutral serum-virus mixture required an entirely different scale of serum and virus concentrations from that required for the precipitin demonstration. The relatively high concentrations of serum and virus that are desirable in precipitin testing are unsuitable for infection tests (4), and, furthermore, mixtures of virus and serum containing no excess antibody or virus from the standpoint of precipitin testing often contain enough free virus to give numerous infections. After some exploratory work, a technique was designed that has regularly demonstrated the recovery of virus from serum-virus mixtures by means of pepsin digestion. The following representative experiment gives the essential features of the technique and results.

The antigenic solution used was a virus-containing tobacco juice that had been concentrated by two precipitations with half-saturated ammonium sulphate, and was about 10 times as infective as virus juice after freezing. This was diluted 1:1000 with saline solution (=1:100 dilution of crude

TABLE 2.—*Recovery of infectivity of neutralized virus as a result of peptic digestion*

Tube	Virus	Serum 1:20	Mg. pepsin P. N./cc.	Hours incubated	Total no. lesions/total no. half-leaves	No. lesions/ half-leaf
1	1:100	Immune	1.0	4	11/17	0.65[a]
2	"	"	"	0	3/17	0.18
3	"	"	0.1	4	16/18	0.90
4	"	"	"	0	7/18	0.40
5	"	"	0.01	4	17/19	0.90
6	"	"	"	0	16/19	0.85
7	"	Normal	1.0	4	111/20	5.60
8	"	"	"	0	163/20	8.20
9	"	"	0.1	4	72/16	4.50
10	"	"	"	0	119/16	7.50
11	"	"	0.01	4	182/18	10.00
12	"	"	"	0	199/18	11.00

[a] These results calculated in table 3.

virus juice). To samples of the diluted virus were added equal parts of purified normal or virus-immune serum at 1 : 20 dilution. These mixtures of serum and virus (with saline solution + virus controls) were shaken and incubated for 2 hours at 37° C. and for 16 hours on ice. Each mixture was then titrated to pH 3 and pepsin was added. Control samples were titrated back to pH 7.0 at once, and test samples were allowed to incubate for 4 hours at 37° C. and were then titrated to pH 7.0. The samples were dialyzed for 16 hours against distilled water, and each sample was tested for infectivity by rubbing on 16–20 half-leaves of *Nicotiana langsdorffii* Weinm. The samples designated 1 and 2 in the tables to follow were rubbed on opposite halves of the same leaves, and similar tests were made with samples 3 and 4, 5 and 6, etc. The results of the experiment are summarized in tables 2 and 3.

It is apparent, when one considers the various substances and treatments involved in this experiment, that many factors contribute toward increasing or decreasing the number of lesions finally obtained. The neutralization of virus by immune serum decreases the lesion count. But the immune serum also contains normal serum proteins that participate in a nonspecific fashion in this decrease of infectivity (4). Besides destroying antibodies, pepsin also has effects on the normal serum inhibition and on the virus directly. The presence of denatured pepsin and pepsin digestion products in the mixtures, the action of the acid on the various constituents, and many other factors, all play a part in determining the number of lesions produced. In an attempt to obtain a somewhat accurate measure of virus recovery, the following method of calculation was employed.

In each test there were 4 observed values, *i.e.:*

A. Number of lesions with virus + normal serum + denatured pepsin;
B. " " " " " + immune " + " " ;
C. " " " " " + normal " + active " ;
D. " " " " " + immune " + " " .

Value A is taken as representing the maximum amount of virus present and potentially infective in any of the 4 serum-virus mixtures (100 per cent). Neutralization without recovery through pepsin action removes the activity of all but $\frac{B}{A} \cdot 100$ per cent of the virus. The action of pepsin in the presence of virus and normal serum decreases the infectivity of the virus by $\frac{C}{A}$, hence $\frac{A}{C}$ is the necessary correction factor for the pepsin-treated immune-serum-virus mixture. The observed percentage of the original virus (A) present in the immune serum-virus mixture after pepsin action is $\frac{D}{A} \cdot 100$, and this,

when corrected for the pepsin-normal serum effect, becomes $\frac{D}{A} \cdot 100 \cdot \frac{A}{C}$. The actual percentage increase in infective virus due to the pepsin effect in liberating neutralized virus, then, is equal to the percentage of free virus after pepsin treatment minus the percentage of free virus before pepsin treatment, or $\left(\frac{D}{A} \cdot 100 \cdot \frac{A}{C}\right) - \left(\frac{B}{A} \cdot 100\right)$, which simplifies to $\left(\frac{D}{C} - \frac{B}{A}\right) \cdot 100$ per cent. This formula was used for calculating the percentages of virus recovery in tables 3 and 4. It is seen from table 3 that with virus at 1:100, immune

TABLE 3.—*Calculation of the data given in table 2*

	Mg. pepsin P. N./cc.		
	1.0	0.1	0.01
A (Lesions, virus + normal serum)	8.20	7.50	11.0
B (" " + immune ")	0.18	0.40	0.85
C (" " + normal serum + pepsin)	5.60	4.50	10.00
D (" " + immune " + ")	0.65	0.90	0.90
Recovery of virus $\left(\frac{D}{C} - \frac{B}{A}\right) \cdot 100\%$[a]	9.4%	14.6%	1.3%

[a] Per cent of neutralized virus in the mixture that was actually liberated through pepsin digestion of neutralizing antibody.

serum at 1:20, and 1 mg. P. N. pepsin acting for 4 hours, the amount of infective virus recovered in the mixture after pepsin action was 9.4 per cent of the maximum theoretical yield. With .01 mg. P. N. pepsin, the recovery of virus was less. It is apparent from the above table that pepsin action on normal serum increases the inhibiting effect of normal serum and, consequently, decreases the number of lesions produced. The pepsin effect on immune serum-virus mixtures includes this action to decrease the number of lesions opposed to the pepsin effect in releasing virus. Because the two effects act in opposite directions, it is necessary to remove the pepsin-normal serum action in order to observe the pepsin-immune serum action adequately. This is accomplished by the correction factor introduced into the formula. The smallest amount of pepsin used had little effect in increasing the nonspecific inhibitory effect of normal serum, while the larger amounts of pepsin increased this effect more markedly.

This type of experiment has been repeated a number of times, and in all cases, unless the amount of pepsin administered was relatively small, a marked recovery of infectivity was noted in the pepsin-immune serum-virus mixtures. Table 4 summarizes the results of these experiments.

TABLE 4.—*Recovery of neutralized virus by pepsin digestion as shown by infectivity tests. Summary of all experiments*

Test	Virus dilution	Serum dilution	Mg.P.N. pepsin per cc.	Pepsin incubation period	Observed number of lesions[a]				Per cent of total virus recovered[a]
					A	B	C	D	
1	1:100	1:20	1.0	4 hrs.	6.1	0.62	5.2	1.50	19.0%
2	"	"	"	"	8.2	0.18	5.6	0.65	9.4%
3	"	1:10	0.2	2 hrs.	8.4	0.40	2.8	0.60	16.6%
4	"	"	"	"	28.0	1.30	6.0	2.00	28.6%
5	"	1:20	0.1	4 hrs.	7.5	0.40	4.5	0.90	14.6%
6	"	1:10	"	"	13.2	4.60	3.9	2.25	23.0%
7	"	"	0.08	5 hrs.	7.7	0.70	1.7	0.90	43.9%
8	"	"	"	"	27.0	0.20	11.0	2.10	18.3%
9	"	"	0.025	4 hrs.	4.0	0.00	5.0	0.20	(100 %)
10	"	"	"	"	16.0	0.20	3.0	0.90	28.7%
11	"	"	0.01	"	10.6	5.20	7.7	3.70	0 %
12	"	1:20	"	"	11.0	0.85	10.0	0.90	1.3%
13	"	1:100	"	5 hrs.	13.0	2.50	3.0	1.30	24.3%
14	"	"	"	"	56.0	8.10	11.0	8.00	58.0%
15	1:1000	"	0.025	2 hrs.	1.8	0.50	1.4	0.40	0 %

[a] For significance of symbols and method of calculating recovery of virus see table 3 and pertinent text.

From this table it is seen that recovery of virus occurred in 13 of 15 tests. In one of the two tests in which recovery was not observed (Test 11), the amount of pepsin was relatively small and the amount of serum to be digested was large, and in the other (Test 15), the virus was at relatively high dilution. On the whole, it appears that these infectivity tests support the precipitin tests in showing the recovery of virus from neutralized virus-serum mixtures. The absolute amounts recovered, as shown by the infectivity tests, are not so large as those recovered when tested by the precipitin method, probably because, under the conditions required for the infectivity tests, the virus is at relatively high dilution and, hence, more subject to pepsin digestion than concentrated virus (13).

It has been held by some serologists that antibodies possess their remarkable specificity because they are merely antigens that have been slightly modified by the serum proteins. If such a view be correct, one might anticipate that virus antigen could be recovered from virus antibodies by partial pepsin digestion of the antibodies. Tobacco-mosaic-immune serum is normally noninfective. Several experiments were performed in an endeavor to demonstrate virus infectivity in such serum after pepsin digestion, but in no case was evidence obtained that virus may be recovered from antibodies alone by pepsin digestion.

The present section has indicated that tobacco-mosaic virus, which has been neutralized by its specific immune serum, may be liberated when the antibodies are destroyed by pepsin digestion. The liberation of virus has been shown both by precipitin and by infectivity tests. This shows that when the virus is neutralized it is not destroyed, but is held in an impotent, noninfective condition from which it may be recovered.

At the time the foregoing studies on tobacco-mosaic virus neutralization were being concluded, a paper appeared by Bawden and Pirie (3) describing studies of the effect of pepsin on the "X" virus (latent mosaic or mottle virus) of potato. They found the "X" virus to be susceptible to pepsin digestion. Antibodies in general are relatively resistant to pepsin, and neutralized tobacco-mosaic virus was recoverable only because it was even more resistant to pepsin than the antibodies. The report that the "X" virus is susceptible to pepsin suggested that pepsin digestion of neutralized "X" virus might liberate antibody rather than virus. Accordingly, experiments were performed to test this possibility.

RECOVERY OF ANTIBODY FROM NEUTRAL ANTISERUM-VIRUS MIXTURES

Johnson's potato-ring-spot virus (a strain of the "X" virus) was employed in the following experiments. The virus was propagated in tobacco and the expressed juice of the tobacco plants was kept frozen until required. Immune serum was prepared in the usual manner, and the antibodies for healthy-tobacco protein were removed by precipitating the serum with 2 volumes of healthy-tobacco juice. Such absorbed serum was kept frozen until required.

In order to determine the optimal proportions of serum and virus for precipitation reaction, various amounts of virus-containing juice were added to serum. In performing the titrations the amount of precipitate formed in the mixtures was recorded, the tubes were centrifuged, and the supernatant fluids tested against antibody and virus to determine the presence, in the mixtures, of excess virus or antibody. The titrations showed that 4 volumes of juice were required to react with all of the antibodies in 1 volume of absorbed serum. Addition of more than 4 volumes of virus juice, however, did not result in the presence of excess virus until 30 or more volumes of virus had been added. In other words, 1 unit of antibody showed the property of reacting completely with 1, 2, 4, or 8 units of virus, but not more. This property of antibody is illustrated in the titrations reported in table 5.

From the table it is seen that with the given amount of virus, addition of more than $\frac{1}{4}$ part of serum resulted in an excess of antibody, while addition of less than 1/32 part of serum resulted in an excess of virus, as shown by the precipitin tests. However, if the constant amount of virus was added

TABLE 5.—*Titration of "X"-virus-containing juice with immune serum*

Tube	Undiluted virus juice (cc.)	Absorbed immune serum		Precipitate	Precipitin test of supernatant fluid with				
		Dilution	cc.		Virus 1:1	Virus 1:10	Serum 1:1	Serum 1:10	
1	1	1:1	1	++[a]	+++	+	0	0	
2	1	1:2	1	++	+	+	0	0	
3	1	1:4	1	++	0	0	0	0	Zone of neutrality
4	1	1:8	1	++	0	0	0	0	
5	1	1:16	1	++	0	0	0	0	
6	1	1:32	1	+	0	0	0	0	
7	1	1:64	1	+	0	0	+	+	
8	1	1:128	1	0	0	0	++	++	
9	1	1:256	1	0	0	0	++	++	
10	1	1:512	1	0	0	0	++	++	

[a] Symbols as in table 1.

to 1/4, 1/8, 1/16, or 1/32 parts of serum, reaction was complete and no excess antibody or virus was detected in the supernatant fluid.

The titrations having shown that 4 volumes of the virus juice would neutralize the antibodies in 1 volume of serum, a large quantity of serum was added to 4 parts of virus-containing juice. The mixture was incubated for 2 hours at 37° C.; the precipitate resulting was centrifuged, washed 3 or 4 times with distilled water, suspended in distilled water, and used in pepsin experiments.

Preliminary experiments showed that, when such precipitate was acidified and treated with pepsin, the virus was destroyed and antibody recovered. However, it soon became apparent that the pepsin was playing little or no part in this reaction, and that acidification alone sufficed to liberate antibody from the precipitates. A representative experiment will be described.

Washed serum-virus precipitate was divided into a series of samples. These were titrated to various pH levels by the addition of HCl. After incubating for 2 hours at 37° C., all of the samples were titrated to pH 7.0, the amounts of undissolved precipitate were noted, the tubes were centrifuged, and the supernatant fluids were tested against immune serum, normal serum, virus, and tobacco juice to detect liberated antibody or virus. The results of these tests are given in table 6.

TABLE 6.—*Recovery of antibody ("X" virus) by acidification of neutral serum-virus precipitate*

Tube	pH	Residual precipitate	Precipitin test of supernatant fluid with			
			Virus 1:1	Immune serum 1:1	Healthy tobacco 1:1	Normal serum 1:1
1	7.0	++[a]	0	0	0	0
2	6.0	++	0	0	0	0
3	5.5	++	0	0	0	0
4	4.8	(+)[b]	++	0	0	0
5	4.0	(+)	++	0	0	0
6	3.0	(+)	++	0	0	0
7	2.5	(+)	++	0	0	0
8	2.0	(+)	++	0	0	0
9	Untreated immune serum[c]		++	0	0	0
10	Untreated virus[c]		0	++	0	0
11	Original supernatant fluid		0	0	0	0
12	Wash fluid 1		0	0	0	0
13	Wash fluid 2		0	0	0	0
14	Wash fluid 3		0	0	0	0
15	Wash fluid 4		0	0	0	0

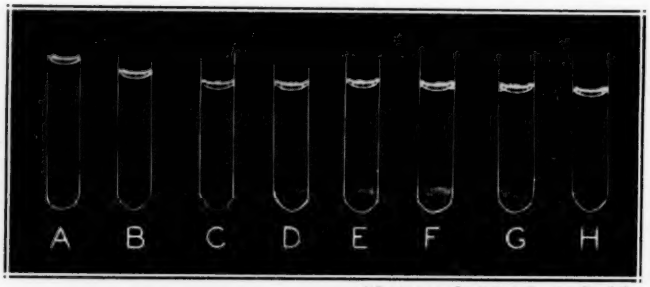
[a] Symbols as in table 1.

[b] Precipitate present but not of the type produced by precipitin reaction. Evidently a precipitate of inactive virus residue.

[c] Adjusted to a concentration equal to that of serum or virus in original precipitate suspension (= maximum possible yield).

From the table it is seen that the precipitate could be taken to pH 5.5 without dissolution of the precipitate or liberation of its constituents. Titration to pH 4.8 to 2.0 resulted in dissolution of the neutral precipitate and the liberation of antibody. The yield of antibody was virtually 100 per cent, as far as could be told by the amount of precipitate when tested against virus (compare tubes 4–8 with tube 9). The accompanying photograph illustrates certain of the tubes of this experiment (Fig. 2). In the photograph, tubes A–C were of the mixtures incubated at pH 7.0, 6.0, and 5.5, respectively, tubes D–G were of the mixtures at pH 4.8, 4.0, 3.0, and 2.0, respectively, and tube H is the control tube (9 in the table), showing the amount of precipitate to be expected, had all of the antibody been recovered.

From experiments of this type, it is concluded that the acidification of neutral "X"-virus-antiserum precipitate at pH 4.8 to 2.0 results in a dissolution of the precipitate and a liberation of large quantities of antibody.



Photographed by J. A. Carlile

FIG. 2. Recovery of "X"-virus antibody by acidification of neutral virus-antibody precipitate.

DISCUSSION

It has been seen in the foregoing pages that the digestion of precipitates of tobacco mosaic and its specific immune serum liberates large quantities of virus, and that the acidification of precipitates of "X" virus and its specific serum liberates large quantities of antibody. From these results it is apparent that in the cases mentioned neutralization of virus by serum does not involve the destruction of either virus or antibody, respectively.

These findings provide serological methods for the purification of either virus or antibody. Such methods differ from the chemical methods usually employed, in that they are much more narrowly specific than chemical techniques for protein fractionation. The methods are not complicated and the yields of virus or antibody are high, of the order of 75 per cent to 100 per cent. It would be very desirable to utilize such methods in the preparation of purified antibody and virus, and then to compare the products obtained with those that have been obtained by the ordinary chemical methods.

Titrations with the "X" virus indicate a considerable versatility of the antibody molecule, since it may combine with and be saturated by any number of units of antibody from 1 to 8. This is in harmony with Bordet's colloidal adsorption theory of toxin-antitoxin neutralization.

SUMMARY

Virus-immune serum, from which the antibodies for healthy-tobacco proteins had been removed, was purified by eliminating from the serum the fraction of protein that was insoluble in 30 per cent saturated ammonium sulphate and that soluble in 43 per cent. The pseudoglobulins rendered insoluble in water by heating to 57° C. also were eliminated. The resulting water-clear fraction after dialysis had suffered little or no loss in virus-antibody content, but showed only a fraction of the nonspecific inhibitory action of unpurified serum. The inhibitory property of normal serum was found to be distributed among all of the protein fractions of the serum.

Neutralized mixtures of tobacco-mosaic virus juice and immune serum were prepared by titrating the serum with the juice until the supernatant fiuid after centrifuging contained an excess of neither serum nor virus, as determined by precipitin testing. When such mixtures were subjected to a number of chemical, physical, and serological treatments, no free virus nor antibody was recovered, but when the mixtures were partially digested with pepsin, the antibodies were destroyed and a large portion of the virus was recovered, as determined by precipitin tests.

Using a somewhat different experimental arrangement, for reasons that are explained, it was possible to show that the partial digestion of mixtures of immune serum and virus with pepsin increased the infectivity of the mixtures through the destruction of antibody and consequent liberation of virus. No infectious matter was obtained by the partial digestion of virus-free immune serum by pepsin, which provides evidence that the virus antibodies are not merely virus particles that have been modified by the serum proteins.

These findings demonstrate that, when tobacco-mosaic virus is neutralized by its specific immune serum, the virus is not destroyed, but is held in an impotent, noninfective condition from which it may be liberated if the antibodies are destroyed by pepsin digestion.

When neutral precipitates of potato "X" virus and its specific serum were acidified to pH 4.8 or below, the precipitate underwent dissolution and large amounts of free antibody were recoverable in the supernatant fluids. This indicates that, in the neutralization of this virus by its specific serum, the antibody is not destroyed.

Titration of "X" virus with its immune serum showed that 1 unit of antibody has the power of combining with and being saturated by any number of units of antigen from 1 to 8.

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LITERATURE CITED

1. ANDREWES, C. H. The action of immune serum on vaccinia and virus III in vitro. Jour. Path. Bact. **31**: 671–698. 1928.
2. ————. Immunity in virus diseases. Lancet, May 2, 1931: 989; May 9, 1931: 1046.
3. BAWDEN, F. C., and N. W. PIRIE. Experiments on the chemical behavior of potato virus "X." Brit. Jour. Exp. Path. **17**: 64–74. 1936.
4. CHESTER, K. S. Specific quantitative neutralization of the viruses of tobacco mosaic, tobacco ring spot, and cucumber mosaic by immune sera. Phytopath. **24**: 1180–1202. 1934.
5. ————. Serological evidence in plant-virus classification. Phytopath. **25**: 686–701. 1935.

6. CHESTER, K. S. The antigenicity of the plant viruses. Phytopath. **25**: 702–714. 1935.
7. HARTLEY, P. Chapter VII. The effect of physical and chemical agencies on the properties of antigens and antibodies. *In:* A system of bacteriology in relation to medicine. Med. Res. Counc. London **1931**: 249–265. 1931.
8. MATSUMOTO, T. Antigenic properties of tobacco mosaic juice. Jour. Soc. Trop. Agr. **1**: 291–300. 1930.
9. MUCKENFUSS, R. S. Studies on the bacteriophage of d'Herelle. XI. An inquiry into the mode of action of antibacteriophage serum. Jour. Exp. Med. **48**: 709–722. 1928.
10. MULVANIA, M. Studies on the nature of the virus of tobacco mosaic. Phytopath. **16**: 853–871. 1926.
11. PURDY, H. A. Immunologic reactions with tobacco mosaic virus. Jour. Exp. Med. **49**: 919–935. 1929.
12. SILBERSCHMIDT, K. Studien zum Nachweis von Antikörpern in Pflanzen. II. Teil B. (Beiträge zur Frage der Resistenz und Immunität von Pflanzen gegenüber dem infizierenden Agens der Viruskrankheiten.) Beitr. Biol. Pfl. **20**: 105–178. 1933.
13. STANLEY, W. M. Chemical studies on the virus of tobacco mosaic. II. The proteolytic action of pepsin. Phytopath. **24**: 1269–1289. 1934.
14. WEISS, E. The bacteriophage-antibacteriophage reaction. Jour. Immunol. **13**: 301–309. 1927.

THE RELATIONSHIP OF CEPHALOSPORIUM ACREMONIUM TO THE BLACK-BUNDLE DISEASE OF CORN

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INTRODUCTION

In fields of corn there frequently are plants that have made an abnormal growth. These abnormalities include barren stalks, nubbin ears, multiple ears at one node, excessive suckering, dwarfing and a purpling or reddening of the leaves. When such abnormal stalks of corn are split open and examined, a part of them will show discolored vascular bundles ranging from light brown to black. (Fig. 1, A). This abnormal condition has been found also in stalks having a normal external appearance and is known as the black-bundle disease of corn. It has been reported from all parts of the United States wherever corn is grown. Recently it has been reported from Kenya Colony, Africa (8). The black-bundle condition has been said to be due to various causes such as genetic factors, (9) environmental factors, and the attacks of fungi, but is most commonly believed to be associated with an invasion of the vascular bundles by the fungus *Cephalosporium acremonium* Corda.

REVIEW OF THE LITERATURE

Symptoms associated with the black-bundle disease of corn have been noted by practical corn breeders in Illinois since 1901. In 1916, Pammel, King, and Seal (6) reported that barren stalks, nubbins, and slow growing stalks were due to an invasion of *Gibberella saubinetii*. In 1920, Norton and Chen (5) studied the black-bundle condition in sweet corn and reported it to be due to an invasion of *Cephalosporium sacchari* Butler and Kahn, which, they then believed, were a Fusarium stage to develop, probably would have to be referred to *Fusarium moniliforme* Sheld. Manns and Adams, (4) in 1921, found *C. sacchari* in kernels of sweet corn and referred to it as a parasitic fungus that could be detected when corn was sprouted on the germinator. Manns, (3) in the same year, reported 39.54 per cent of all the seed corn in Delaware was infected by *C. sacchari*. Black bundles in the nodal area were said by Hoffer and Carr (1) to be due to phosphorus deficiency in the soil, which resulted in an accumulation of iron and aluminum salts in the nodal plate and the bundles passing through it.

[1] The writer is indebted to Dr. J. G. Dickson of the University of Wisconsin for helpful advice and criticism during the progress of the work; to Dr. James R. Holbert for cooperation and assistance in field studies at Bloomington, Illinois; and to the Funk Bros. Seed Company for the use of their experimental plots.

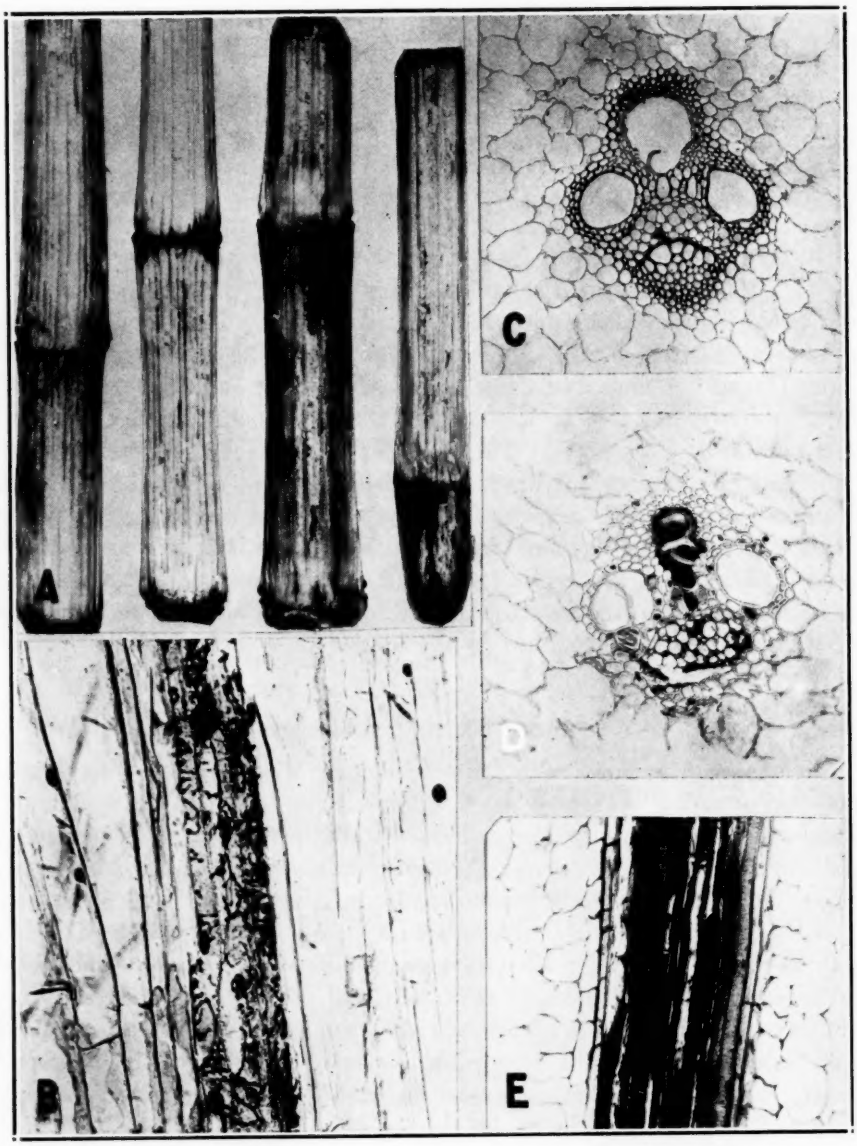

FIG. 1. A. Longitudinal sections through cornstalks showing discolored vascular bundles. B. Longitudinal section of a seedling corn-plant root invaded through an injured end by *Cephalosporium acremonium*. C. Cross-section of a normal vascular bundle of corn. D. Cross-section of a vascular bundle filled with gum. E. Longitudinal section of a vascular bundle filled with gum.

The most extensive study of the black-bundle disease of corn was published by Reddy and Holbert (7), in 1924, They found that the blackened vascular bundles, in plate cultures, very often produced *Cephalosporium acremonium*, which was mistaken by Manns and Adams for *C. sacchari*. Reddy and Holbert reported that the blackened condition of the bundles was due to an invasion of that fungus. They associated many corn abnormalities, such as multiple ears at one node and excessive sucker production, with the presence of black bundles. They reported that the fungus was seed-borne and completed its life cycle within the host. They stated that they demonstrated definitely the pathogenicity of the organism by injecting a spore suspension into the plant by means of a hypodermic needle. In 1930, Koehler and Holbert (2) in an extensive bulletin on the corn diseases of Illinois, listed the black-bundle disease. They stated that the black-bundle condition usually is caused by an invasion of *C. acremonium*, but that there is a possibility of its being caused by other factors. In 1935, Thorold reported from Kenya Colony, Africa, that the black-bundle disease was found there. He stated that *C. acremonium* was not always associated with the black-bundle condition (8).

METHODS OF EXPERIMENTATION AND DATA OBTAINED

In order to determine fully the relationship between the black-bundle condition and the fungus *Cephalosporium acremonium*, two methods of experimentation were used. First, corn plants showing the presence of black bundles in the field under natural conditions were examined, plated, and portions of the black bundles were fixed in formol acetic alcohol. Later, pieces of this fixed material were imbedded in paraffin, sectioned, and stained for microscopic study. Second, corn plants both in the field and in the greenhouse, were inoculated with cultures of the fungus in an effort to produce blackened bundles and abnormal growth characters. Along with this work, the effect of unfavorable environmental factors was noted as an influence in the production of black bundles.

THE BLACK-BUNDLE CONDITION

From January, 1928, to June, 1933, a study was made of the occurrence of the black-bundle condition. Experiments were carried on in the winter in the greenhouse and in the summer in the field. Several inbred strains of corn were used that have shown black bundles when grown under varying field conditions. These strains were developed by J. R. Holbert, Bloomington, Illinois, and have been inbred several years. One of these strains, known by the symbol A1237, has been under observation in this study for 5 years at Madison, Wisconsin; Bloomington, Illinois; and Sacramento, California. In 1928, this strain was grown at Madison, along with 26 other inbred strains,

and when examined at the end of the season, was found to have a large proportion of dark to black vascular bundles. This condition in the A1237 strain has been observed in every stalk examined during this study.

In general, the bundles were not a dense black but varied from light to deep brown. When a stalk was cut in cross section at the center of the internode, the darkened bundles usually were few in number. More were found in the lower than in the upper internodes. When viewed in longitudinal section, these bundles were found to vary in color in the same internode. Usually, they were darkest near the node and grew progressively lighter toward the center of the internode. Sharp variations in intensity of color frequently were noted in the same bundle. For one or two centimeters it would be very dark and abruptly would show scarcely any coloration. Actual counts showed that the number of darkened bundles per internode varied considerably. It was found that they were more numerous in the lower than in the upper internodes. In some of the stalks it was found that the darkened bundles were fewer in the lower than in the higher internodes. Strain A1237 was observed as having a darker discoloration of the bundles when grown on phosphorus-deficient soils showing excess nitrogen. On such soils the strain exhibited such abnormalities as decreased vigor of stalk growth, flamed and colored leaves, and no ears. This condition has been general in this strain grown under these conditions. The consistent appearance of the black bundles in this strain, regardless of where it is grown, suggested that the black-bundle condition was associated with hereditary tendencies.

Other strains of corn have been observed to have blackened bundles at times. Under the severe drouth conditions of 1930, in central Illinois, four other inbred strains of corn were found that, when examined, showed the black-bundle condition. These strains are known by the symbols BR10, AR9, G, and Illinois Low Ear. During years of normal soil moisture, these strains rarely showed black bundles, and have not shown the black-bundle condition when grown on soils deficient in phosphorus.

RESULTS OF PLATING OF BLACK BUNDLES

When it had been definitely demonstrated that black bundles occur regularly in the A1237 strain of corn, correlation of the presence of the fungus *Cephalosporium acremonium* with this condition was attempted, as has been reported by Reddy and Holbert (7). A series of platings was begun when the plants were about a foot high and completed after the stalks were mature. Black bundles were found in the stalks during this entire period. Internodes that were to be plated were cut from the stalks and freed of leaves and sheaths. They were washed in bichloride of mercury, split lengthwise with a sterilized scalpel, and a portion of the blackened bundles removed aseptically to a sterile test tube containing potato-dextrose agar.

The majority of the black bundles plated did not contain active cultures of *C. acremonium*, for less than 4 per cent of the cultures yielded the fungus. (Table 1.) Later, additional platings from the A1237 strain were made. Out of a total of 174 plants examined, slightly more than 4 per cent produced cultures on agar plates. When averaged in with the data presented in table 1, exactly 4 per cent of 343 stalks of the A1237 strain produced cultures of *C. acremonium*.

TABLE 1.—*Summary of platings of roots and black bundles of corn from June 21, 1929, to October 8, 1929*[1]

Date of platings	Part of the plant	Cultures from platings			
		Cephalosporium acremonium	Other fungi	Free of fungi	Totals
June 21, 1929	Roots	0	0	10	10
27, 1929	"	0	0	10	10
July, 9, 1929	Internodes	0	0	10	10
16, 1929	"	0	0	10	10
23, 1929	"	3	0	17	20
Aug. 2, 1929	"	0	0	5	5
11, 1929	"	0	0	4	4
18, 1929	"	0	0	20	20
29, 1929	"	0	0	20	20
Sept. 19, 1929	"	3	8	9	20
24, 1929	"	0	4	16	20
Oct. 8, 1929	"	0	0	20	20
Totals		6	12	151	169
Per cent		3.8	7.5	88.7	100

[1] Platings made at Madison, Wisconsin.

Black bundles from strains other than A1237 were plated and found to contain an occasional trace of the fungus. Platings were made from the inbred strains BR10, AR9, G, and Illinois Low Ear, in 1930, when these strains developed blackened bundles. Out of a total of 77 stalks examined, less than 4 per cent were infested with the fungus. In summarizing the data of all the platings of black bundles it was found that 3.9 per cent of the 420 stalks of inbred corn were infested with active *Cephalosporium acremonium*. Nine and five-tenths per cent showed cultures of a number of other fungi, the one occurring most often being *Diplodia zeae* (Schw.) Lev. Of all the stalks examined, 86.6 per cent were fungus-free.

HISTOLOGICAL STUDIES

Darkened vascular bundles from which the fungus was plated, together with sterile bundles, were fixed, imbedded, sectioned, and stained with Dela-

field's haematoxylin and counter-stained with orange G in clove oil. The fungus mycelium stained a deep purple. Sections of bundles, known to be free of the fungus, showed a gum-like substance deposited in the cells (Fig. 1, D – E), which remained unstained. This substance ranged from light yellow to very dark brown. In bundles showing light deposits, the gum-like substance was located in the sieve tubes and companion cells of the phloem. In the more heavily impregnated bundles, the xylem vessels were often plugged and, in extreme cases, all of the cells of the bundle were filled with gum. In sections where the fungus was present, the mycelium was confined almost entirely to the xylem vessels and closely associated with the gum. Occasionally, hyphae were observed to pass through vessel pits into other cells of the bundle. Frequently, the fungus formed a mat of mycelium on one side of a vessel wall, but was never observed completely plugging a vessel. In plants grown under field conditions the fungus always was found associated with bundles containing some of the gum-like substance.

MICROCHEMICAL STUDIES

Microchemical studies on fresh and fixed materials showed the gum to be of a complex chemical nature. The material was fixed in formol-acetic alcohol and then dehydrated. It was then imbedded in paraffin, sectioned, cleared in xylol, and placed in absolute alcohol. It was then ready for the microchemical test as outlined by Eckerson. For fixed tissue the test is as follows:[2]

> Put sections on a slide in one per cent. phloroglucin solution. (0.1 gram phloroglucin; 10.0 c.c. alcohol); add a drop of concentrated hydrochloric acid and observe. Lignin immediately turns violet. Heat carefully for about ten minutes. Pentoses become cherry red.

The pale yellow gum-like substance gave a cherry red reaction. The darker portions of the gum were not specific in their color reaction.

Fresh material was tested for pentoses with orcin. Freehand sections, cut from the internodes of newly harvested corn stalks, were kept in sterile distilled water until tested. The fresh sections were placed in a 4 per cent orcin solution. The excess solution was drained off with filter paper, and a drop of concentrated hydrochloric acid added. According to Eckerson,[2] lignin becomes immediately violet, without heating; the pentoses a blue to violet, after heating. The lighter colored gum deposits sometimes showed the blue or violet color, and at other times remained the original yellow, indicating considerable variation in composition. The darker portions of the gum always remained a deep brown and were not in any way acted upon by the chemicals used. In both fixed and fresh materials these tests would

[2] Eckerson, Sofia H. Microchemistry. [Unpublished mimeographed manuscript of the Department of Botany, University of Chicago.]

indicate that substances of a pentose nature were sometimes associated with the gum-like deposits.

Varied tests were run to determine the relative solubility of the mass of gum. When freshly cut freehand sections were put into 0.5 per cent solution of ammonium oxylate, and the solution heated to the boiling point, a noticeable dissolving of the lighter colored gum occurred. When these same sections were treated further with a 4 per cent solution of sulphuric acid, slightly more of the lighter portions of the gum were dissolved out. The residual material, and especially the darker colored mass of the deposit, was not hydrolysed when heated in strong acids at high temperatures.

FACTORS FAVORING GUM PRODUCTION

During the progress of the field study it was observed that corn plants of strain A1237, when grown in soil low in available phosphorus and having an excess of nitrogen, always contained more of the black bundles than were found in plants of this strain grown in a balanced fertile soil. In contrast, the BR10 strain did not react the same on the phosphorus deficient soil; but during the drouth in 1930, it did show black bundles. The results were checked in greenhouse experiments. The two strains of corn were grown in white sand in culture cans to which a balanced and an unbalanced nutrient solution were added by substituting KNO_3 for KH_2PO_4 in the control.[3] Culture cans with normal soil, deficient in moisture, also were used.

Both strains of corn behaved under greenhouse conditions as they did in the field. The results (Table 2), show that black bundles were produced in strain BR10 when it was grown under drouth conditions but not when grown under conditions of unbalanced nutriment. Just the opposite effect was obtained with strain A1237, which produced fewer black bundles than usual on dry soil, but did produce numerous black bundles on an unbalanced nutrient solution. These results indicate that the presence of black bundles in the inbred lines of corn studied may be produced by different environmental changes, with a specific reaction in different inbred lines.

TABLE 2.—*Growth of two strains of corn under varying nutritional and moisture conditions with reference to the presence of the black-bundle condition*

Conditions of growth	Strain of corn	
	A1237 bundles	BR10 bundles
In moist soil	Lightly discolored	No discoloration
In dry soil	Very lightly discolored	Heavily discolored
Balanced nutrient solution	Lightly discolored	No discoloration
Unbalanced nutrient solution	Heavily discolored	No discoloration

[3] Detmer's nutrient solution: Water, 1000 g.; $Ca(NO_3)_2$, 1.00 g.; KCl, 0.25 g.; $MgSO_4$, 0.25 g.; KH_2PO_4, 0.25 g.; $FeCl_3$, trace.

THE ORGANISM FOUND ASSOCIATED WITH BLACK BUNDLES

The fungus isolated from the small percentage of the black bundles and classified as *Cephalosporium acremonium* corresponds with that described by Reddy and Holbert (7) as the cause of the disease. The colonies of the fungus are orbicular, dense, floccose, with the mycelium at first very thin, hyaline, branched, and sparsely septate. The conidiophores are erect, simple, and arise laterally from vegetative hyphae. They are uniseptate and 40 μ to 60 μ high. The conidia are numerous, elliptical or oblong, straight or curved, and nearly hyaline. They are very small (4.0×1.5 μ) and are borne singly at the apex of conidiophores. Each spore is pressed to one side by the next spore produced and they all adhere, by means of a slime, forming a head. The spore heads easily fall apart when submerged in water and the spores are freed.

When grown on different media, colonies of the fungus vary greatly in macroscopic characters. Some colonies are resupinate and creep along the surface of the medium. Others have a dense, felt-like mycelium, or coremium-like structures. Some colonies tend to assume a spiral growth to the right or left. Cultures do not remain constant in their characters, but over a period of time through several transfers they may show all the described variations of macroscopic appearance. At the lower temperatures, the fungus is white, but at higher temperatures it may become a delicate rose pink. It makes its best growth at relatively high temperatures. Petri-dish cultures were incubated for 2 weeks at temperatures ranging from 16° to 36° C., and the rate of growth was determined by the diameter of the colonies. It was found that the optimum temperature for mycelial growth was about 30° C.

The fungus grows well on any medium containing an available source of carbon. It makes a vigorous growth on potato-water agar containing a 6-carbon sugar, such as dextrose, lactose, galactose, maltose, and saccharose. It makes a similar growth when dextrin is the source of carbon. On medium containing the 5-carbon sugar xylose, the fungus made a growth similar to that on any of the hexose sugars. The growth on xylan, isolated from corn cobs, was twice that made in the same time on the hexose sugars. Growth was vigorous on corn meal-dextrose agar, and oatmeal agar media. A lesser growth was made on nutrient agar, bile agar, and agar containing Richard's solution. The fungus developed little aerial hyphae on these last 3 media, the cultures having much the same appearance as cultures of bacteria.

INOCULATION STUDIES IN THE GREENHOUSE

Several methods of inoculation with the organism were used on young and older corn plants grown in the greenhouse. After the roots of seedlings

had been injured, to give the fungus a possible means of entry, the seedlings were placed in soil containing the organism. Older plants were inoculated by pouring a spore suspension over the uninjured roots, and over the roots injured in various ways (Fig. 2). The most extensive type of inoculation

FIG. 2. A. Two plants of strain A1237 inoculated by pouring spore suspension of *Cephalosporium acremonium* over severely injured roots. B. Two plants of strain L111 inoculated as in A.

was carried on with a hypodermic needle. The fungus was cultured on agar and washed to obtain a spore suspension. This suspension was then hypodermically injected into the stalk of the young plant. In addition to these

methods of inoculation, plants grown from naturally infected seed were observed for disease symptoms and cultured to determine the possible presence of the fungus.

Corn seedlings were inoculated with the fungus through root injuries and then carried to maturity. Kernels of strain A1237 were surface-sterilized and then grown on agar. Those kernels, free of fungi and bacteria, when the seedlings had reached the four-leaf stage of development, were transferred to large test tubes containing sterile soil. The young plants continued to develop normally in these tubes for 2 weeks. They were then removed from the tubes and the ends of the roots were injured with a scalpel. They were transplanted to sterilized soil that had been inoculated with a spore suspension of *Cephalosporium acremonium* (Fig. 1, B). After ten days in this soil, several injured root ends from each plant were plated. The plants were carried to maturity. All of the root ends plated produced the fungus; but out of 20 of these plants, which were carried to maturity, no cultures of the fungus were plated from the vascular bundles.

Corn plants growing in soil to which a heavy spore suspension had been added at varying intervals during the growth of the plant reached maturity without infection. Corn plants having darkened bundles, such as strain A1237, became infected when the roots were injured at the time the plants reached the silking stage. No infection resulted from wounded roots being exposed to the fungus prior to silking. Plants that did not show the black-bundle condition were not invaded by the fungus through root injuries at any time during the life of the plant. The data on this series of root injury inoculations are given in table 3.

TABLE 3.—*Summary of data on root inoculation experiments in the greenhouse*

Stage of growth when inoculated	Strain	Cultures from platings			
		Cephalosporium acremonium	Other fungi	Free of fungi	Totals
6th leaf stage	A1237	0	22	108	130
Tassels showing	A1237	0	0	75	75
Tassels showing	BR10	0	0	75	75
Silk showing	A1237	20	0	0	20
Silk showing	BR10	0	0	10	10
Silk showing	L111	0	0	10	10

There appeared to be no difference in the relative pathogenicity of 6 isolations of the fungus. Macroscopically, these cultures varied considerably. Two of the isolations were made at Bloomington, Illinois, 2 at Massillon, Ohio, and 2 at Madison, Wisconsin. Spore suspensions of the 6 isolations were

inoculated into plants when 2 feet high by means of a hypodermic needle. The strain BR10 was inoculated. The fungus was recovered from every stalk inoculated. Externally, the plants appeared to be normal, and the fungus progressed but a very short distance from the point of inoculation.

Reddy and Holbert (7) reported that infected ears carry the organism internally in the seed; that the fungus develops with the germinating kernel and causes a systemic infection of the plant through the vascular system; and by this means, invades the ears and eventually the kernels. They believed that in this manner the fungus is carried over to the following season. A study of this phase of their work failed to bear out their findings.

Seed infected with *Cephalosporium acremonium*, but free of all other fungi and bacteria, was secured by plating from infected ears. Kernels were surface-sterilized, plated on potato dextrose agar, and only those with a pure culture of the fungus growing out of them were used. The seedlings were planted in sterilized soil and grown to maturity. Three out of 30 such stalks, when plated at maturity, yielded cultures of the fungus from the bundles. It was not possible to determine whether these 3 stalks were infected when the kernels germinated or whether infection took place some time later during their growth. All stalks were of strain A1237 and contained dark bundles.

FIELD INOCULATIONS

In order to determine the effect of an invasion of the fungus on field-grown corn plants, inoculations were made with a hypodermic needle. At Madison, Wisconsin, 3 strains of corn, A1237, BR10, and L111, were inoculated hypodermically. One hundred plants of each strain, approximately a foot high, were inoculated with a spore suspension. One hundred check plants of each strain were given a hypodermic injection of distilled water. Two hundred plants of each strain, in the silking stage, were given a hypodermic injection of the spore suspension. An equal number of uninjured plants of each strain, growing adjacent to the inoculated strains, served as controls. When examined in the field under conditions of normal growth and without inoculation with the fungus, strain A1237 always showed black bundles; strain BR10 showed black bundles occasionally, and strain L111 rarely, if ever, showed the black-bundle condition.

Externally, strain A1237 alone showed any effects of inoculation. A very small proportion of these stalks, inoculated when a foot high, appeared to be stunted in the same way as those described by Reddy and Holbert (7). However, as much stunting appeared among the controls injected with sterile distilled water. In other strains, the inoculation made no difference in the external appearance of the stalks. Of all the plants inoculated when a foot high, only those of strain A1237 showed black bundles. In the strains inoculated at the appearance of silks, the bundles of strains BR10 and L111 were

black a few inches away from the point of injury, but the stalks as a whole were normal in appearance. As shown in table 4, 410 platings were made in which there were 65 *Cephalosporium acremonium* cultures and 364 were free of fungi. Five and one-tenth per cent of the early inoculations and 34.5 per cent of the late inoculations were effective. It is interesting to note that in strain L111 26 out of 30 stalks were found to contain active cultures of the fungus, but the areas infected in the stalks were restricted to within 2 inches of the point of inoculation. The fungus did not grow beyond these areas, and very small areas within the stalk were damaged by the puncture and the action of the fungus.

TABLE 4.—*Summary of data on inoculations made with a hypodermic needle and a spore suspension of Cephalosporium acremonium under field conditions at Madison, Wisconsin—season of 1930*

Platings listed by strains	Inoculation method				
	Spore suspension July 12	Water check	Spore suspension August 1	Check	Totals
A1237					
C. acremonium	5	1	11	1	18
Free of fungi	93	20	32	148	303
BR10					
C. acremonium	—	—	21	—	21
Free of fungi	—	—	48	—	48
L111					
C. acremonium	—	—	26	—	26
Free of fungi	—	—	4	—	4

At Bloomington, Illinois, a large number of hybrid and inbred strains of corn were inoculated with a spore suspension of the fungus. This was done when the tassels began to show. Two plots were used. Treatments were alike, except that the checks in the first plot were not given the sterile distilled water injection. When mature, a large number of stalks were split open and examined (Table 5). In this series, including more than 5,000 plants, it was noted that *Diplodia zeae* attacked many stalks inoculated with a spore suspension of *Cephalosporium acremonium* and also those injected with sterile distilled water, entering through the wound made by the hypodermic needle. The stalks not attacked by *D. zeae* and that had been inoculated with *C. acremoninum* showed no outward symptoms of disease at the end of the season. The drouth was severe and greatly reduced the yield of corn, but *C. acremonium* seemed to react on the plants in the same

way as it did at Madison, Wisconsin, where similar experimental plots were grown to maturity under normal soil-moisture conditions. No platings were made in this series, the experiment being designed to bring out the external symptoms, which have been described by other workers as being due to an inoculation with the fungus. No external symptoms of disease appeared and thus the experiment checked with other inoculation experiments in which the organism was recovered.

TABLE 5.—*Showing the influence of inoculation with a spore suspension of Cephalosporium acremonium into corn plants grown at Bloomington, Illinois—season of 1930*

Data on inoculation series and check series	Number of stalks examined		
	Plot No. 279	Plot No. 283	
	Inoculation	Inoculation	Control
Inoculated with *Cephalosporium acremonium*	1776	1785	—
Injected with sterile distilled water for control purposes	—	—	1774
Examined for black bundles by splitting open the stalks	712	897	839
Bundles blackened by *C. acremonium* inoculation only	351	277	—
Bundles blackened by an invasion of *Diplodia zeae* through wounds made by hypodermic needle	314	376	427
Bundles blackened as a result of the response of a number of strains to severe drouth conditions[1]	47	226	170
Bundles white, apparently normal, and not damaged by the sterile water injection	—	—	242
Average distance bundles were blackened due only to an inoculation of *C. acremonium* spores	7.8 in.	7.7 in.	—

[1] Under normal environmental conditions in which there is no soil-moisture deficiency, most of the strains recorded in this group do not have black bundles.

DISCUSSION

In this study it has been shown that the black-bundle condition of corn is found consistently in some inbred strains, occasionally in some, and is totally lacking in others. Strain A1237 is consistent in having some discolored bundles in every stalk. Strain L111 is consistently free of any discoloration of the bundles. Some inbred strains of corn have darkened bundles when grown under adverse environmental conditions, while, under normal growing conditions, no black bundles occur.

Since Reddy and Holbert (7) named the fungus *Cephalosporium acremonium* as the causal fungus of the black-bundle disease, a study was made of the occurrence of the fungus in the black bundles found in strain A1237 and in the occasional stalks of other strains showing this condition.

The results show conclusively that, in those inbred strains of corn studied, the presence of the fungus in the bundles is not a factor in bringing about the black-bundle condition, only 4 per cent of the bundles being infested with the fungus.

A histological study of black bundles showed that the cells, particularly those of the protoxylem and phloem, were filled with a dark, amorphous material, ranging in color from a light brown to black. This material was studied microchemically in both fixed and fresh sections. While the results are inconclusive, enough was learned of the nature of the substance to demonstrate that it contains, in part, pentoses.

The gum-like substance in the bundles was evidently either an environmental response of the plant or due to a hereditary factor in strains such as A1237, where it occurs in every stalk. An effort was made to produce the black-bundle condition in strains which were normally free of it. It was demonstrated that darkened bundles could be produced either by drouth conditions or an unbalanced nutrient condition in inbred strains, where they do not normally occur. Later, this was again demonstrated under field conditions by the inbred strain BR10. It is normally free of vascular discoloration, but showed many black bundles in each stalk when grown under the drouth conditions of the year 1930.

Since the fungus *Cephalosporium acremonium* was occasionally found in black bundles, several attempts were made to inoculate corn plants with the fungus. Seedlings were grown in infested soil. The roots of the primary root system were injured, and ten days later the fungus had penetrated several centimeters up the vascular bundles of these roots. However, at maturity only 10 per cent of the plants thus invaded were infested with the fungus in the vascular bundles of the stalks. Thus it is demonstrated that plants invaded by the fungus in the seedling roots may continue growth to maturity without the fungus penetrating to other portions of the stalk.

When spores of the fungus were hypodermically injected into the vascular bundles of various strains of inbred corn and the plants brought to maturity, no differences could be noted in any strain except A1237, which has black bundles consistently. In strain A1237, wounding by the hypodermic needle caused stunting, both in plants injected with sterile water and in plants inoculated with a spore suspension of the fungus. The fungus, in all strains except A1237, remained alive in the wounded bundles until the stalks had matured, but had progressed only a very short distance from the point of inoculation.

When cultured on artificial media, the fungus grew most vigorously on medium containing xylan isolated from corn cobs. In the vascular bundles of corn stalks free of the brown gum-like substance, the fungus made no ap-

preciable growth. In bundles where the gum was present the fungus grew vigorously, if able to penetrate through wounds to the gum-filled bundles. The results of the study suggest that *Cephalosporium acremonium* is not a vigorous pathogenic fungus, but rather is it a saprophyte that occasionally is able to penetrate through wounds to vascular bundles already partially filled with a gum-like substance offering food material to the fungus.

SUMMARY

Previous workers have described the black-bundle disease of corn as being caused by invasion of the vascular bundles by the fungus *Cephalosporium acremonium*. Abnormal external growth features were ascribed to hereditary factors, improper nutrition, and the invasion of the fungus *C. acremonium*.

In this study the blackening of the vascular bundles in certain inbred strains was found to be due to a deposit of gum-like material in the cells and the vessels of the bundle. In a small percentage of cases the fungus was present and found to be closely associated with the gum. Preliminary microchemical analysis of the gum indicates the presence of small amounts of pentoses, which serve as food for the fungus.

The deposit of gum in the vascular bundles was found associated with hereditary characters in one inbred strain of corn, as well as being influenced by environmental conditions. Black bundles were produced under controlled environmental conditions by limiting the water supply of plants of one strain of corn and by supplying an unbalanced nutrient solution to plants of another strain. Under field conditions, black bundles were observed to occur in some strains during the severe drouth of 1930. In another strain, soils deficient in phosphorus were found to cause black bundles.

Inoculation experiments with corn seedlings of the several inbred strains under observation did not result in infection. Planting infected seed did not result in the establishment of the fungus in the growing plant. Older plants, nearing maturity, from strains of corn having vascular bundles filled with a gum-like substance were found to be invaded by the fungus. Strains of corn having bundles free of the gum-like substances were not invaded by the fungus. Infection of those strains from which the fungus was cultured was found to have taken place after the corn stalks had tasseled out and following severe root injury. In none of the experiments made was the fungus found to be an active pathogen invading normal vascular bundles through uninjured root systems.

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LITERATURE CITED

1. HOFFER, G. N., and R. H. CARR. Accumulation of aluminum and iron compounds in corn plants and its probable relation to rootrots. Jour. Agr. Research [U. S.] **23**: 801–824. 1923.
2. KOEHLER, B., and J. R. HOLBERT. Corn diseases in Illinois, their extent, nature, and control. Illinois Agr. Expt. Sta. Bull. 354. 1930.
3. MANNS, T. F. Report of the Department of Plant Pathology and Soil Bacteriology. Delaware Agr. Expt. Sta. Bull. 129: 18–30. 1921.
4. ————, and J. F. ADAMS. Prevalence and distribution of fungi internal of seed corn. Science (n.s.) **54**: 385–387. 1921.
5. NORTON, J. B. S., and C. C. CHEN. Another corn seed parasite. Science (n.s.) **52**: 250–251. 1920.
6. PAMMEL, L. H., C. M. KING, and J. L. SEAL. Studies on a fusarium disease of corn and sorghum. (Preliminary). Iowa Agr. Expt. Sta. Research Bull. 33: 111–136. 1916.
7. REDDY, C. S., and J. R. HOLBERT. The black-bundle disease of corn. Jour. Agr. Research [U. S.] **27**: 177–206. 1924.
8. THOROLD, C. A. Diseases of cereal crops in Kenya Colony. Kenya Colony. Dept. Agr. Bull. 2. 1935.
9. WENTZ, J. B., and S. F. GOODSELL. Recessive defects and yield in corn. Jour. Agr. Research [U. S.] **38**: 505–510. 1929.

LEAF BLIGHT OF CHINA ASTER CAUSED BY RHIZOCTONIA SOLANI[1]

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INTRODUCTION

During the autumn of 1935 a considerable number of China aster plants (*Callistephus chinensis* Nees) growing under greenhouse conditions at Princeton, New Jersey, exhibited a severe blighting of the leaves. A fungus identified as *Rhizoctonia solani* Kühn was isolated from the diseased leaf tissue, and its pathogenicity was established by pure-culture inoculation.

While it is well known that *Rhizoctonia solani* has a wide host range, and that plants may be attacked at different stages of development (8), a study of the literature indicates no report of the fungus as causing a leaf blight of China aster. Duggar and Stewart (2) have reported *R. solani* responsible for stem lesions on mature China aster plants, and Peltier (8) has shown the fungus to be capable of causing damping off of the seedlings of this host. Direct attack of leaves by *R. solani* has been pointed out, by both Duggar (1) and Matsumoto (6), to be infrequent. Examples of leaf diseases caused by this fungus have been described by Stone and Smith (10) for lettuce, and by Wellman (11) for cabbage.

Whether or not this isolate of *Rhizoctonia solani* was peculiar in its ability to attack the leaves of China aster was not known. A study was, therefore, undertaken to compare this isolate with others of the same species, but collected from different hosts. The present paper deals with the results of these comparative studies.

THE DISEASE

The disease was first noticed in the early autumn of 1935 on 2- to 4-month-old plants during and shortly following an extended period of warm, cloudy weather. As the season advanced and the temperature lowered, a decrease in incidence of blighting became evident.

Symptoms of the disease first appeared on the lowermost leaves, which had opportunity of coming in contact with the soil. Affected tissues were characterized by a clearly defined, water-soaked area that progressed rapidly over the leaf. Within a short time the infected area collapsed and became

[1] Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by the date of receipt of the manuscript. This practice in nowise delays the publication of manuscripts printed at the expense of The American Phytopathological Society or other agency.

flaccid, turning from a deep green to a dead brown. More or less concentric zones of alternating light and dark brown were often observed spreading from an infection center. Following collapse of the leaf blade, the infection spread down the petiole to the stem, where other leaves became involved. Severely blighted leaves often showed scattered strands of coarse mycelium traversing their surfaces. Under favorable conditions for development of the disease, plants frequently were killed within a period of a few days (Fig. 1).

Photographed by J. A. Carlile.

FIG. 1. The China aster plant on the left shows severe infection by *Rhizoctonia solani*. The plant on the right is healthy.

EXPERIMENTAL

In order to determine whether the isolate obtained from diseased China asters was similar to or markedly different from isolates of *Rhizoctonia solani* from other hosts, a number of comparative experiments were undertaken. The experiments consisted of studies on growth rates at different temperatures, growth and cultural behavior on various agar media, cross inoculations, and modes of penetration. An outline of the history of the 5 isolates of *R. solani* employed is given in table 1.

Temperature Relationships. Each isolate was grown in triplicate on plates of potato-dextrose agar at each of 7 different temperatures. Mycelium used in seeding the plates was cut in equal amounts from the marginal growth of 4-day-old colonies grown on the same medium at the respective temperatures. In the case of those isolates that failed to grow at the extreme temperatures, mycelium used to seed the plates was taken from colonies grown at the next closest temperature. Daily measurements were taken of the diameters of the colonies for 3 succeeding days after placing the cul-

TABLE 1.—*History of isolates of Rhizoctonia solani used in the experiments*

Isolate	Host from which isolated	Geographical origin of isolate	Date of isolation
A–1	China aster	Princeton, N. J.	1935
P–1	Potato (Sclerotium)	"	"
SB–1[a]	Sugar beet	Minnesota	1934
SB–2	"	"	1930
SB–3	"	Michigan	"

[a] The strains isolated from sugar beets were furnished to the writer through courtesy of Dr. E. L. LeClerg.

tures at the various temperatures. Growth curves for the isolates at the end of the 3rd day at the different temperatures are shown in figure 2, and are based on the average of 3 replications of the experiment.

Isolate A–1 showed no growth at 5° C., very little at 10° C., and an optimum growth temperature between 25° and 30° C. The outstanding feature is the amount of growth produced by this isolate at 35° C. Isolate SB–3, although making much better growth at the lower temperatures than A–1, was the only other culture to show appreciable growth at 35° C. Isolates SB–1 and SB–2 showed a close similarity in temperature relationship, and were intermediate in this respect between P–1 at the low temperatures, and A–1 at the higher temperatures. The culture P–1 grew better at comparatively low temperatures and showed a pronounced reduction in colony diameter above 25°.

Growth on Different Agar Media. In this experiment the isolates were grown in triplicate at room temperature (22°–24° C.) on the following agar media: malt, prune, cornmeal, lima-bean, potato-dextrose, oatmeal, Richard's, and Coon's.[2] Mycelium used to seed the plates was cut in equal amounts from the marginal growth of 4-day-old colonies growing on each of the media. The diameters of the colonies were measured at 24-hour intervals for a period of 3 days.

The data in table 2 show Coon's agar to be the most favorable medium

[2] The malt, prune, cornmeal, and Lima-bean media were prepared "Difco" agars. Potato-dextrose agar was made as follows: 200 g. of peeled potatoes were steamed for ½ hour in 500 cc. distilled water. To the filtered potato juice 20 g. of dextrose were added, and 500 cc. of melted 4 per cent agar were then mixed with the potato juice to bring the final agar concentration to 2 per cent. The mixture was made up to volume (1 liter) and autoclaved 20 minutes at 20 lbs. pressure. The synthetic media were of the following composition: Richard's solution agar: sucrose, 50 g.; magnesium sulphate, 2.50 g.; monobasic potassium phosphate, 5.00 g.; potassium nitrate, 10 g.; agar, 20 g.; distilled water, 1000 cc. Coon's medium agar: sucrose, 7.20 g.; dextrose, 3.60 g.; magnesium sulphate, 1.23 g.; monobasic potassium phosphate, 2.72 g.; potassium nitrate, 2.02 g.; agar, 20 g.; distilled water, 1000 cc.

FIG. 2. Graph of growth curves of 5 isolates of *Rhizoctonia solani* at 7 different temperatures at end of 3 days.

△ = Isolate A–1
□ = Isolate P–1
▲ = Isolate SB–3
■ = Isolate SB–1
○ = Isolate SB–2

for the growth of isolate SB–3. This medium also supported the best growth in the case of isolate A–1. While not expressed in the table, readings of the colony diameters of this isolate on Coon's agar on the 1st and 2nd days

were greater than on the other media. Oatmeal agar proved to be the most favorable medium for the growth of cultures SB–1, SB–2, and P–1. In this experiment, as well as that on temperature relations, a similarity in behavior was found between isolates A–1 and SB–3 and between the isolates SB–1 and SB–2.

TABLE 2.—*Growth in millimeters of the 5 isolates of Rhizoctonia solani on different agar media*

Isolate	Colony diameters in millimeters on 8 agar media at the end of 3 days							
	Malt	Prune	Corn-meal	Lima-bean	Potato dextrose	Oatmeal	Richard's	Coon's
A–1	85	57	78	90 +[a]	90 +	90 +	65	90 +
P–1	48	46	60	77	80	90 +	40	52
SB–1	44	20	25	63	58	90 +	29	38
SB–2	45	33	42	82	64	84	30	44
SB–3	63	72	79	90	82	81	80	90 +

[a] The + sign indicates that the colonies had overgrown the entire agar surface.

Comparisons were made of the cultural behavior of the 5 isolates *Rhizoctonia solani* to determine possible similarities or differences between them. The following is a description of the cultural characters of each isolate grown for 7 days at 22°–24° C. on potato-dextrose agar:

A–1. Sclerotia irregular in size and shape, sparse over the surface of the colony, but abundant about the edge and in contact with glass wall of the Petri plate. Aerial mycelium abundant, closely compressed to surface of agar, and showing radiating lines of mycelial growth. Color of colony: olive buff.

P–1. Sclerotia irregular in size and shape, aggregated in clumps in center of colony but diminishing in abundance towards the edge. Aerial mycelium moderately abundant and woolly in appearance. Color of colony: olive brown, fading in intensity towards edge.

SB–1. Sclerotia irregular in size and shape, evenly distributed over entire colony. Aerial mycelium abundant and woolly in appearance. Color of colony: sepia brown, of equal intensity over entire colony.

SB–2. Sclerotia irregular in size and shape, evenly distributed over the surface of the colony. Aerial mycelium abundant and woolly in appearance. Color of colony: tawny olive.

SB–3. Sclerotia very sparse and small. Aerial mycelium abundant, closely compressed to surface of agar and showing radiating lines of growth. Color of colony: pale olive buff.

In general, the isolates A–1 and SB–3 show a close similarity in cultural behavior, as do also isolates SB–1 and SB–2. Isolate P–1, although resembling SB–1 and SB–2 in many respects, differs markedly in the abundance and aggregation of sclerotia.

Cross inoculations. Young plants of China aster, sugar beet (*Beta vulgaris* L.) and potato (*Solanum tuberosum* L.) were inoculated with each of the 5 isolates by placing pieces of mycelium of equal size on the leaves and stem. Inoculum was held in place by covering with pieces of moist absorbent cotton. Controls consisted of plants to which only the moist absorbent cotton was applied. The plants were placed in a moist chamber for 48 hours, after which they were removed to a greenhouse bench. Two days later, observations were made on the pathogenicity of the isolates on the 3 hosts. A summary of the results is given in table 3.

TABLE 3.—*Relative pathogenicity of the 5 isolates of Rhizoctonia solani on 3 hosts*

Isolate	Relative degree of pathogenicity		
	Sugar beet	China aster	Potato
A–1	Very severe infection	Very severe infection	Moderate infection
P–1	No infection	No infection	No infection
SB–1	Moderate infection	Severe infection	Moderate infection
SB–2	" "	" "	" "
SB–3	Severe infection	" "	" "

Each isolate was relatively uniform in its behavior on a given host. No infections occurred in the noninoculated controls. The aster isolate, A–1, was unquestionably the most pathogenic of all cultures employed. Sugar beet plants inoculated with this isolate were characterized by severe infection that spread from the point of inoculation and involved most of the aboveground parts. The same general condition held for China aster plants inoculated with this isolate. Potato plants, while showing distinct symptoms, proved to be much more resistant to all isolates than did the other 2 hosts.

The potato isolate, P–1, was found to be nonpathogenic under the conditions of the experiment.

Of the cultures SB–1, SB–2, and SB–3, the latter was slightly more pathogenic on sugar beets and China asters than the other 2.

Penetration Studies. During the course of the investigation, the question arose as to whether or not the nonpathogenic isolate, P–1, penetrated the tissue of the inoculated plant. It was desired also to determine the modes of penetration of the other isolates.

The plants used in these studies were China aster, sugar beet, and begonia (*Begonia semperflorens* Link and Otto). Since the stomata of this species

of begonia are found only on the lower surface of the leaves, it afforded a means of studying penetration both in the absence and in the presence of these natural openings.

The leaves were inoculated on both surfaces and the plants placed in a moist chamber. In addition, leaves that had been wounded with a series of pin punctures also were inoculated. At 24-hour intervals, leaves, each of which was inoculated with a single isolate, were removed and pieces of tissue cut out from around the place of inoculation. The pieces of tissue were placed in a solution of equal parts of absolute alcohol and glacial acetic acid to remove the chlorophyll, then cleared in lactophenol, and finally stained in lactophenol to which acid fuchsin had been added.

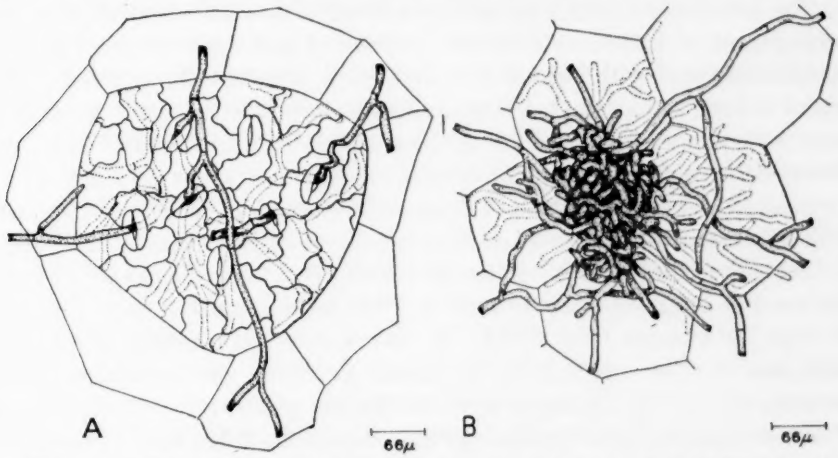

FIG. 3. A, Hyphae penetrating through stomata on lower surface of Begonia leaf. This was found to be the characteristic mode of penetration of cultures SB–1 and SB–2. B, "Infection cushion" on upper surface of Begonia leaf, by means of which hyphae of cultures A–1 and SB–3 are able to penetrate cuticularized epidermal cells.

The material fixed 24 hours after inoculation showed no evidence of penetration by any of the fungus cultures. After 48 hours, infection was macroscopically evident on leaves inoculated with isolates A–1 and SB–3. Microscopic observation showed the mycelium traversing the surface of the leaves and small stromatic areas of knotted fungus strands distributed among the hyphae (Fig. 3, B). The stromatic areas or "infection cushions" have been described already by Duggar (1) as playing an important rôle in effecting penetration.

Initial penetration by the isolates A–1 and SB–3 takes place immediately beneath infection cushions and seems to be a mechanical process. The infection cushions appear to act as a fulcrum against which a force is exerted that is sufficient to allow hyphae to penetrate uninjured, cuticularized epi-

dermal cells. Infection cushions were formed on the lower as well as the upper surface of all inoculated leaves, regardless of the location of stomata.

The exact means by which the infection cushions are held on the surface of the host, to permit a force to act on them without changing their position, was not determined. No cementing material was ever observed around the cushions. Furthermore, the cushions could easily be lifted from the leaves by means of a fine glass needle, suggesting that their weight may be sufficiently great to balance the pressure exerted by the penetrating hyphae. Swelling of the ends of the hyphae in contact with the cell walls, and bulging of the latter in the direction of the force exerted, suggest that the progress of the fungus within the host tissue is largely a mechanical process.

The inoculated plants were held in a moist chamber for several days and development of infections followed. Wounded and nonwounded leaves of plants inoculated with isolates A–1 and SB–3 were heavily infected at the end of 5 days. Nonwounded Begonia leaves, inoculated on their upper surfaces with isolates SB–1 and SB–2, showed no infection at the end of 7 days. Wounded leaves of all plants, nonwounded leaves of sugar beet and China aster, and nonwounded leaves of Begonia inoculated on their lower surfaces with SB–1 and SB–2 showed marked symptoms of infection within 5 days.

Leaves inoculated with isolates SB–1 and SB–2 showed evidence of penetration and infection after 48 hours. Penetration occurred, however, only through the stomata (Fig. 3, A). In no instance was penetration found to take place through the uninjured cuticle. Infection was established by all isolates, except P–1, in leaves wounded by pin punctures. The hyphae of P–1 were observed growing through and about cells killed by the punctures, but subsequent penetration of living cells was never observed.

DISCUSSION

The formation of infection cushions and their rôle in effecting direct penetration appear to be only partially responsible for the high degree of pathogenicity shown by isolates A–1 and SB–3. That the ability of a given culture to penetrate directly does not seem to be the sole criterion for determining pathogenicity is suggested by the fact that certain cultures, namely, SB–1 and SB–2, do not form these infection cushions, but gain entrance into the living tissue only through stomata. Furthermore, the virulence of a culture appears to depend on more than simple penetration, since in the case of isolate P–1, where hyphae entered the host through wounds, subsequent penetration and parasitism of living cells did not take place. It is probable that in nature the most highly pathogenic cultures of *Rhizoctonia solani* penetrate directly and are not dependent on wounds or stomata as avenues of entrance into their hosts.

In addition to their very marked pathogenicity and mode of host penetration, the cultures A–1 and SB–3 could be differentiated from cultures P–1, SB–1, and SB–2 on the basis of cultural characters, growth on agar media, and temperature relationships. Cultures A–1 and SB–3 could be distinguished from each other primarily on the basis of their growth rates at 35° C.

Although isolates of *Rhizoctonia solani*, such as A–1, may have very well defined characteristics that serve to distinguish them one from another at a given time, the continued stability of such characters seems somewhat doubtful. From time to time during the course of the work, all isolates gave rise to one or more sectorial variants differing in cultural behavior from the original parent culture. The variants, however, were not studied in any detail, and whenever such sectors arose the original culture was segregated and maintained separately. The fact that many different types of cultures of *R. solani* are encountered on original isolation, suggests that variants may arise as frequently under natural conditions as in artificial culture.

The presence of definite strains within the species *Rhizoctonia solani* has been recognized by several investigators, namely, Matsumoto (6), Edson and Shapovalov (3), Rosenbaum and Shapovalov (9), Gratz (4), and LeClerg (5). These strains have been distinguished on the basis of pathogenicity, and on their physiological and cultural characters while being maintained on artificial media.

Peltier (8), and Monteith and Dahl (7), on the other hand, have pointed out that the fungus is highly variable and specialization towards particular hosts is not sufficiently distinct to warrant a definite classification of strains.

In any fungus species like *Rhizoctonia solani*, in which the mycelium is multinucleate and uninucleate spore forms are not produced in artificial culture, the possibility of the existence of a heterocaryotic thallus cannot be overlooked. Where heterocaryosis exists in the original isolates, attempts to delimit them into highly specialized strains of stable characters would appear to present many difficulties.

SUMMARY

A leaf blight of China aster caused by *Rhizoctonia solani* is reported and symptoms of the disease described.

The culture of the fungus, A–1, isolated from diseased aster leaves, has been shown to differ in its physiology, cultural characters, pathogenicity, and mode of host penetration from certain other cultures (SB–1, SB–2, and P–1) of the same species. Similarity in certain characters was shown to exist between the culture A–1 and the culture SB–3 isolated from sugar beets.

No marked host specialization was found to exist among any of the isolates tested.

The cultures A–1 and SB–3 produced infection cushions on the surface of the host which function in direct penetration of the cuticle. Cultures SB–1 and SB–2 were observed to penetrate only through stomata without the intervention of infection cushions. The culture P–1 did not penetrate the living tissue of any of the plants inoculated, even though the latter had been wounded and the hyphae were in contact with the exposed noncuticularized tissue.

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LITERATURE CITED

1. DUGGAR, B. M. *Rhizoctonia crocorum* (Pers.) DC. and *R. solani* Kühn (*Corticium vagum* B. and C.) with notes on other species. Ann. Mo. Bot. Gard. **2**: 403–458. 1915.
2. ————, and F. C. STEWART. The sterile fungus *Rhizoctonia solani* as a cause of plant diseases in America. N. Y. (Cornell) Agr. Expt. Sta. Bul. **186**: 51–76. 1901.
3. EDSON, H. A., and M. SHAPOVALOV. Potato stem lesions. Jour. Agr. Res. **14**: 213–220. 1918.
4. GRATZ, L. O. Wire stem of cabbage. N. Y. (Cornell) Agr. Expt. Sta. Mem. **85**. 1925.
5. LECLERG, E. L. Parasitism of *Rhizoctonia solani* on sugar beet. Jour. Agr. Res. **49**: 407–431. 1934.
6. MATSUMOTO, T. Studies in the physiology of the fungi. XII. Physiological specialization in *Rhizoctonia solani* Kühn. Ann. Mo. Bot. Gard. **2**: 1–62. 1921.
7. MONTEITH, J., JR., and A. S. DAHL. A comparison of some strains of *Rhizoctonia solani* in culture. Jour. Agr. Res. **36**: 897–903. 1928.
8. PELTIER, G. L. Parasitic Rhizoctonias in America. Ill. Agr. Expt. Sta. Bul. **186**: 283–390. 1916.
9. ROSENBAUM, J., and M. SHAPOVALOV. A new strain of *Rhizoctonia solani* on the potato. Jour. Agr. Res. **9**: 413–420. 1917.
10. STONE, G. E., and R. E. SMITH. The rotting of greenhouse lettuce. Mass. (Hatch) Sta. Bul. **69**. 1900.
11. WELLMAN, F. L. Rhizoctonia bottom rot and head rot of cabbage. Jour. Agr. Res. **45**: 461–469. 1932.

ANALYSIS OF TYPICAL PLANT DISEASES FROM THE QUARANTINE STANDPOINT[1]

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In estimating the worth of plant quarantines as a means of excluding injurious plant diseases in a permanent way, the logical procedure would be to get together a comprehensive body of information on the foreign parasites against which quarantine action would be directed; then, by studying these in the light of the quarantine measures available for protection, it should be possible to arrive at some general evaluation of the worth of a plant quarantine system for exclusion purposes.

Unfortunately, such an orderly method of approach is seriously hampered at the outset by lack of requisite information on the foreign parasites directly concerned. In many cases the casual agent is new, or has been only slightly studied; references in literature are scanty at times; language difficulties interfere with complete understanding; and the record is too often incomplete from the plant quarantine standpoint. It is a not uncommon experience to encounter a descriptive article that may be highly satisfactory to the mycologist but gives no hint of the very points necessary in appraising the disease from the quarantine viewpoint.

Because of these and other difficulties incident to a direct approach to the problem, an indirect method has been adopted. An attempt has been made to subject to analysis a selected list of 200 more or less typical plant diseases, so as to bring out the relations of their life habits and hosts to accepted quarantine procedure. If we may then assume that these diseases represent in a general way the perplexing field of plant diseases with which quarantine measures would have to deal, the conclusions that may be drawn from them as to the value of quarantines for exclusion purposes ought to hold fairly well in regard to the whole field of plant diseases.

Recognizing that in this indirect method of approach the value of the conclusions depends wholly on the faithfulness with which the substitute list represents the whole field of plant diseases, considerable care has been taken with the examples chosen. In addition to giving consideration only to diseases of outstanding character, an effort has been made to so select the examples that they may be typical, not only of the general types of disease commonly met, such as rots, wilts, rusts, leaf spots, etc., but also so that the list would include a considerable diversity of parasitic species and a varied

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range of hosts. Although most of the hosts because of this method of selection are plants of some economic importance, the economic status of the host has been entirely disregarded in the analysis and the appropriate quarantine action has been derived in each case solely from the biological relationships of host and parasite.

For the sake of simplicity exclusion is here thought of ideally in connection with some sufficient natural barrier, such as a sea, desert, or mountain range, across which materials must be transported by human agency to effect distribution of the disease. The question of exclusion from an adjacent portion of the same land area is not specifically dealt with, though, to the extent that this more difficult and complicated problem involves the transport of disease-carrying materials, the conclusions will be generally applicable.

In this analysis there had to be faced the possibility of the transport of spores or other living stages of parasites on all sorts of commodities other than the hosts themselves and articles directly connected with these hosts. It is quite possible, for example, that spores of *Graphium ulmi* might be carried on such things as azalea plants, or corn smut spores on rye straw packing. But, short of stopping all international interchange of goods, there appears to be no way of avoiding this risk; it simply must be accepted. Consequently, there has been adopted a conservative attitude that disregards incidental spore transportation unless the host material under consideration has had special or definite opportunity to become a carrier. From this viewpoint potato seeds are regarded as free from spores of *Corticium;* but bean seeds, very likely to become contaminated by spores of *Cercospora cruenta* in threshing operations, are treated as carriers of that fungus.

In this study four types of quarantine action are recognized—*embargo, detention, disinfection,* and *inspection.* The addition of unrestricted entry gives us five methods of disposal for imported plant materials. *Embargo,* as the term is universally understood, involves an absolute prohibition on the entry of specific materials. *Detention* is here considered to mean a delay in release during which period the plant materials are held under conditions precluding the escape of parasites, and under careful observation, until freedom from the parasite is assured, or the organism has been eliminated, or until the development of infection compels destruction. Detention must be interpreted as including all holding conditions appropriate for the particular disease concerned, and might range from daily scrutiny in a closely constructed greenhouse to mere field culture under proper observation. It would likewise include the sorting, inspection, and disinfection which would naturally be associated with this means of obtaining disease-free plant products. *Disinfection* also is used in a broad sense to include the considerable number of treatments regarded as effective in freeing various plant materials from their living parasites. Inspection methods and safe holding pro-

visions are of course natural elements in such procedure. *Inspection* is here used in its commonly accepted sense, with the meaning that the plant materials will be examined carefully by a competent official, and if objectionable disease conditions be found, appropriate action will be taken, such as refusal of entry, requirement of treatment, or destruction of the material. Inspection throughout this analysis is considered as a distinct quarantine function only when it alone is depended on as a means of protection; when used as an adjunct to detention or disinfection it is regarded merely as a part of that procedure.

It may be observed that certification of products in the country of origin has not been accorded a sixth place among the quarantine methods at our disposal. This feature was not omitted out of any disrespect for a highly important element in the world's protective arrangements. But, where complete exclusion of a disease is aimed at, could certification, as customarily practiced, be depended on as a sole and only means of protection? Is there anywhere a system of certification so accurate and infallible that it could be trusted to the utmost in the matter of protecting another country's vital interests? No doubt in a few special cases such confidence would be justified, but over the general field of diseases we are forced to conclude that certification, however valuable it may be otherwise, can be considered only as a helpful auxiliary to exclusion measures, or as a partial substitute for them.

The accompanying tabular statement presents separately the indicated quarantine action for seeds, propagating materials other than seeds, and commercial products. It is believed that this division of plant imports enables us to grasp the quarantine relationships with a closer approach to actuality than would any other method. Seeds as a group exhibit characteristic differences from plants, bulbs, or roots, in their concentrated volume, a considerable natural freedom from infection, the usually dormant condition of organisms present, and inability to withstand treatment. Commercial products, on the other hand, develop at times special problems as compared with seeds and other plant propagating materials, because of such factors as their large volume, wide distribution, the presence of active stages of parasites, susceptibility to treatment injury, and the undesirability of using poisonous fungicides on various food products. The results of the analysis seem to substantiate the correctness of this classification. One could perhaps reasonably expect still more fruitful results if these categories were subdivided further, but that refinement is beyond the scope of the present simple analysis.

Turning attention to the tabulated results, it is seen that the 200 diseases fall into 16 categories representing as many types of disease. By this segregation on a roughly symptomatic rather than systematic basis, it was

hoped to uncover any possible differences among these disease types. Any marked differences would have considerable practical significance in actual quarantine procedure. Within each disease type were assembled as varied a series of causal agents as possible.

Glancing now at the summarized totals it is apparent that for 199 seeds (including fern spores) embargo is scarcely needed (0 cases); detention would be required in but few cases (8); disinfection seems to be highly important (116); inspection has but little value (1); and unrestricted entry could be permitted in an encouragingly large proportion (74).

In materials for propagation other than seeds, which occur in 132 of the 200 examples, the picture is not so favorable. It is true that in only 12 cases embargo would seem to be necessary; but 86 would require detention, and in only 29 the more feasible disinfection procedure could be relied on. For a disappointingly small number, inspection (4), and unrestricted entry (2), are seen to be satisfactory.

Commercial products present a most discouraging feature in the large proportion of cases where embargo is indicated if exclusion is sought. One hundred ninety-four of the examples involve products that might be of this type and it is rather disturbing to note that embargo is indicated as a necessary measure in 144 of these instances. Of these 144, 20 cases involve cut flowers, 20 concern straw, important from the quarantine point of view on account of its universal use as packing, and 9 deal with hay. Eighty-three however relate to grain, fruits, vegetables, and similar products entering into commerce in often large quantities. In 10 cases the product affected may be classed as of minor importance.

Even if we disregard cut flowers and the 10 items of minor importance, there still remain 112 cases out of 194 where an exclusion program would have to consider embargo; and it is probable that this proportion would be further increased by the necessity of resorting to embargo in a good many cases where disinfection is indicated as a correct enough biological procedure, but where its use would be accompanied by troublesome difficulties. In this connection one might mention the disinfection of table potatoes to eliminate Rhizoctonia, or of wheat to destroy the internal stage of bunt, *Tilletia tritici*. The outlook for disinfection for these products is, likewise, not very promising, since there are only 31 instances where it could be used effectively. Inspection makes a slightly better showing than in the other two categories, but the mere 12 cases where inspection alone is deemed effective give this function a very unimportant rank. In 56 cases at least some article of commercial type could be allowed unrestricted entry with safety.

As far as these 200 diseases can be considered representative, their analysis indicates that in any serious effort to exclude plant diseases three outstanding quarantine functions must be called into frequent use. In the case of

seeds disinfection would be of preeminent value; detention would be highly important for other propagating materials; and for commercial products embargo would have most frequent use. Disinfection seems to have a rather limited application in the case of propagating materials other than seeds and for commercial products, while detention methods appear to be of quite secondary importance for seeds. The very low percentage of cases in all three categories where inspection alone can be relied on for disease-exclusion purposes suggests that this phase of quarantine activity should not be given undue importance in a system of quarantine aimed at exclusion of diseases.

If we attempt to translate the results of this analysis into terms of quarantine policy, the most disconcerting aspect seems to be the large scale on which embargo of commercial products would have to be considered as the price of adequate disease exclusion. It is true that the actual extent of embargo might be less than the recorded percentage of cases would indicate, since the embargo in many of the cases cited is but a partial one. On the other hand, it is almost certain that an administration would in some cases be driven by necessity to resort to embargo where a less drastic procedure is biologically correct, but, for one reason or another, can not be made effective. We must take into consideration also the very important fact that many diseases involve a much wider host range than the single one that, for the sake of simplicity, has here been associated with the parasite. Less than 60 of the 200 diseases listed are restricted to one host or to a few closely related plants. The remaining 140 have such numerous hosts that any attempt to use embargo freely and consistently throughout this group must promptly and profoundly reduce imports. In over 40 cases the number of hosts is so great that embargo for these is almost out of the question.

It would appear, therefore, that the chief limiting factor in setting up an ideal plant-protection system will be found in connection with commercial products rather than in seeds and other propagating materials. We are sometimes inclined to stress the seriousness of the risk in propagating materials in the matter of disease introduction, and in one sense this attitude is correct. For reasons obvious to every plant pathologist these materials are extremely dangerous, at least potentially. But this analysis seems to indicate that, in a broad way, methods are available that can be employed effectively to provide for safe entry of a very generous proportion of these necessary means of propagation. An efficient protective system, liberal enough for a country's healthy horticultural development, and well within the limits of public tolerance, is seemingly quite attainable for seeds and propagating stock, even if the ideal cannot always be reached. But in connection with commercial products this hopeful outlook is lacking. Any system of protection aiming at a complete program of disease exclusion based on correct biological principles would be compelled at the outset to reduce imports of

commercial plant products to a decidedly embarrassing if not dangerous degree, and probably much beyond the point of popular tolerance.

In this predicament a compromise system would appear to be inevitable. The ideal itself is beyond the reach of every country with considerable foreign interests. How close to perfection can the compromise be established? We may suppose that the standard will vary for different situations. It is quite understandable that a disease-exclusion policy that might serve for an essentially industrial nation importing large quantities of needed plant products would be considered quite inadequate for a country devoted primarily to agriculture, or that exclusion measures judged to be adequate for a one-crop nation would fall far short of the protection it would be profitable to establish in a land with many highly diversified crops.

In the average case it is probable that the compromise would settle down into a policy involving at the least an earnest attempt to exclude specific diseases likely to be destructive to crops of national importance, and in addition, an endeavor to keep out as many other diseases as possible by exclusion measures that may be effected without undue disturbance to the national economy.

SUMMARY

An analysis of 200 typical plant diseases from the quarantine standpoint indicates the quarantine action (embargo, detention, disinfection, inspection, and unrestricted entry), which would be necessary for seeds, propagating materials other than seeds, and commercial products, of the chief host, if exclusion of these diseases is aimed at. Summarized results indicate that embargo is scarcely needed for seeds, would have a minor use for other propagating materials, but would be necessary on a large scale for commercial products. Comparatively few seeds would require detention, but for other propagating materials detention would be freely used. Disinfection, the important procedure for seeds, is only moderately useful for other propagating materials and for commercial products. Inspection of itself has in all three categories a very low rank for exclusion purposes. Many seeds, but very little other propagating material, are safe for unrestricted entry; a rather limited number of commercial products are safe for such entry. The limiting factor in an ideal exclusion system appears to lie in the field of commercial products rather than in propagating materials; for the latter effective quarantine protection is practically attainable, but the need for excessive embargo in the case of commercial products tends to restrict quarantine action to a compromise system far short of perfection.

TABLE 1.—*Analysis of 200 typical plant diseases indicating relation of quarantine procedures to exclusion*

No.	Host	Causal organism	Seeds					Propagating material other than seeds					Commercial products				
			Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry
		MOLDS															
1	Apple	Leptothyrium pomi					x			x						x	
2	Onion	Colletotrichum circinans					x		x				x				
3	Orange	Capnodium citri			x				x				x				
		ANTHRACNOSE, FRUIT SPOTS, SCABS, etc.															
4	Apple	Cylindrosporium pomi			x					x			x				
5	Apple	Venturia inaequalis					x		x							x	
6	Bean	Colletotrichum lindemuthianum		x									x				
7	Canteloupe	Cladosporium cucumerinum			x								x				
8	Clover	Colletotrichum trifolii			x								x				
9	Cotton	Glomerella gossypii			x										x		x
10	Currant	Pseudopeziza ribis			x				x				x				
11	Grape	Sphaceloma ampelinum			x				x				x				
12	Lemon	Sphaceloma fawcettii			x				x				x				
13	Lima Bean	Elsinoe phaseoli			x								x				x
14	Peach	Cladosporium carpophilum			x				x				x				
15	Pecan	Fusicladium effusum			x				x						x		
16	Potato	Actinomyces scabies					x			x					x		
17	Potato	Spongospora subterranea					x			x					x		
18	Raspberry	Plectodiscella veneta			x				x				x				
19	Snapdragon	Colletotrichum antirrhini			x			x					x				
20	Sugar-cane	Colletotrichum falcatum					x		x				x		x		
21	Sweet potato	Cystospora batata					x			x					x		
22	Watermelon	Colletotrichum lagenarium			x								x				

TABLE 1.—(*Continued*)

No.	Host	Causal organism	Seeds					Propagating material other than seeds					Commercial products				
			Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry
		LEAF SPOTS															
23	Alfalfa	Pseudopeziza medicaginis			x								x				
24	Azalea	Septoria azaleae			x				x								
25	Bean	Cercospora cruenta			x								x				
26	Beet	Cercospora beticola			x				x				x				
27	Bluegrass	Phyllachora graminis			x								x				
28	Celery	Septoria apii			x			x					x				
29	Cherry	Coccomyces hiemalis					x		x				x				
30	Clover	Phyllachora trifolii			x								x				
31	Corn	Physoderma zea-maydis			x								x		x		
32	Horseradish	Ramularia armoraciae			x				x							x	
33	Maple	Rhytisma acerinum					x				x						x
34	Oat	Helminthosporium avenae			x								x				
35	Peanut	Cercospora personata			x								x				
36	Pine	Lophodermium pinastri			x				x								x
37	Poplar	Marssonia populi			x				x								x
38	Quince	Fabraea maculata			x				x							x	
39	Rice	Piricularia oryzae			x								x				x
40	Rose	Diplocarpon rosae					x		x				x				
41	Strawberry	Mycosphaerella fragariae			x				x				x				
42	Tobacco	Bacterium angulatum			x								x				
43	Tomato	Septoria lycopersici			x			x					x				
44	Turnip	Albugo candida			x				x				x			x	
45	Violet	Alternaria violae			x				x				x				

TABLE 1.—(*Continued*)

No.	Host	Causal organism	Seeds					Propagating material other than seeds					Commercial products				
			Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry
		BLIGHTS AND CANKERS															
46	Alfalfa	Bacterium medicaginis			x								x				
47	Apple	Phyllosticta solitaria			x				x							x	
48	Apple	Nummularia discreta					x			x							x
49	Apple	Sphaeropsis malorum			x				x				x				
50	Box	Nectria rousselliana			x				x								
51	Chestnut	Endothia parasitica			x				x						x		x
52	Red Cedar	Phomopsis juniperovorus					x		x								x
53	Oak	Strumella coryneoides			x				x				x				x
54	Orange	Bacterium citri			x				x				x				
55	Pea	Ascochyta pisi		x									x				
56	Poplar	Dothichiza populea			x				x				x				x
57	Raspberry	Leptosphaeria coniothyrium			x				x				x				
58	Rose	Diaporthe umbrina					x		x				x				
59	Sycamore	Gloeosporium nervisequum			x				x				x				x
60	Walnut	Pseudomonas juglandis			x				x				x		x		x
61	Willow	Cytospora chrysosperma			x				x				x				x
62	Willow	Fusicladium saliciperdum			x				x				x				x

TABLE 1.—(*Continued*)

No.	Host	Causal organism	Seeds					Propagating material other than seeds					Commercial products				
			Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry
							WILTS										
63	Barberry	Verticillium albo-atrum			x				x								
64	Cabbage	Fusarium conglutinans			x			x					x				
65	Cucumber	Bacillus tracheiphilus			x								x				
66	Elm	Graphium ulmi			x				x				x				x
67	Grape	Cryptosporella viticola					x		x								x
68	Maple	Verticillium sp.			x			x					x				
69	Potato	Fusarium oxysporum					x		x				x				
70	Sweet potato	Fusarium batatatis			x				x				x				
71	Tomato	Fusarium lycopersici			x			x					x				
							ROTS										
72	Apple	Cephalothecium roseum			x					x			x				
73	Apple	Glomerella cingulata			x				x				x				
74	Cabbage	Bacterium campestre			x								x				
75	Corn	Diplodia zeae		x									x				
76	Cranberry	Pestalozzia guepini			x					x			x				
77	Cucumber	Pythium aphanidermatum					x									x	
78	Iris (bulb)	Penicillium sp.			x				x				x				
79	Lemon	Pythiacystis citrophthora			x					x			x				
80	Onion	Botrytis allii					x			x			x				
81	Orange	Penicillium digitatum			x					x			x				
82	Peach	Rhizopus nigricans			x					x			x				
83	Peach	Sclerotinia fructicola			x					x			x				
84	Potato	Fusarium trichothecioides					x		x				x				
85	Potato	Phytophthora infestans					x		x				x				
86	Sweet potato	Botrytis cinerea					x		x				x				

TABLE 1.—(*Continued*)

No.	Host	Causal organism	Seeds					Propagating material other than seeds					Commercial products				
			Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry
		WOOD ROTS															
87	Fir	Trametes pini					x					x			x		
88	Maple	Fomes applanatus					x				x				x		
89	Oak	Armillaria mellea					x	x							x		
90	Pine	Fomes officinalis					x					x			x		
		WITCHES' BROOMS, GALLS, KNOTS, etc.															
91	Alfalfa	Urophlyctis alfalfae			x								x				
92	Apple	Bacterium tumefaciens					x		x								x
93	Blackberry	Fusarium rubi			x				x				x				
94	Cabbage	Plasmodiophora brassicae					x									x	
95	Cherry	Exoascus cerasi					x		x								x
96	Cherry	Plowrightia morbosa					x		x								x
97	Cranberry	Synchytrium vaccinii					x		x				x				
98	Hickory	Microstroma juglandis			x				x								x
99	Oak	Taphrina coerulescens			x					x							x
100	Olive	Pseudomonas savastanoi			x				x								x
101	Peach	Exoascus deformans			x				x				x				
102	Plum	Exoascus pruni					x		x								x
103	Potato	Chrysophlyctis endobiotica					x		x				x				
104	Rhododendron	Exobasidium vaccinii			x				x								

TABLE 1.—(*Continued*)

No.	Host	Causal organism	Seeds					Propagating material other than seeds					Commercial products				
			Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry
		POWDERY MILDEWS															
105	Barley	Erysiphe graminis			x								x		x		
106	Dahlia	Erysiphe cichoracearum			x					x			x				
107	Corylus	Phyllactinia corylea			x					x							x
108	Gooseberry	Sphaerotheca mors-uvae					x			x			x				
109	Grape	Uncinula necator					x			x			x				
110	Lilac	Microsphaera alni					x			x			x				
111	Pea	Erysiphe polygoni			x								x				x
112	Rose	Sphaerotheca pannosa					x			x			x				
113	Willow	Uncinula salicis			x					x			x				x
		DOWNY MILDEWS															
114	Cabbage	Peronospora parasitica			x				x				x				
115	Corn	Sclerospora graminicola			x								x		x		
116	Cucumber	Pseudoperonospora cubensis			x								x				
117	Grape	Plasmopara viticola					x			x			x				
118	Hop	Pseudoperonospora humuli			x				x						x		
119	Lettuce	Bremia lactucae			x								x				
120	Onion	Peronospora schleideni			x					x			x				
121	Tobacco	Peronospora tabacina			x										x		x
122	Wheat	Sclerospora macrospora			x								x		x		x

TABLE 1.—(*Continued*)

No.	Host	Causal organism	Seeds					Propagating material other than seeds					Commercial products				
			Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry
								RUSTS									
123	Apple	Gymnosporangium juniperi-virginianae					x				x					x	
124	Asparagus	Puccinia asparagi			x				x								x
125	Aster	Coleosporium solidaginis					x		x				x				
126	Bean	Uromyces appendiculatus			x								x		x		
127	Carnation	Uromyces caryophyllinus			x				x				x				
128	Clover	Uromyces trifolli			x								x				
129	Flax	Melampsora lini			x								x		x		
130	Hollyhock	Puccinia malvacearum			x				x								
131	Pine	Cronartium cerebrum					x		x								x
132	Plum	Puccinia pruni-spinosae			x				x				x				
133	Raspberry	Gymnoconia interstitialis					x		x								x
134	Rose	Phragmidium subcorticum					x		x				x				
135	Snapdragon	Puccinia antirrhini			x				x				x				
136	White Pine	Cronartium ribicola					x	x					x				x
137	Wheat	Puccinia graminis			x								x		x		x
								SMUTS, etc.									
138	Bamboo	Ustilago shiraiana			x				x				x				x
139	Barley	Ustilago hordei			x								x		x		
140	Colchicum	Urocystis colchici			x				x				x				
141	Corn	Ustilago zeae			x								x		x		
142	Millet	Sorosporium syntherismae			x								x		x		

TABLE 1.—(*Continued*)

No.	Host	Causal organism	Seeds					Propagating material other than seeds					Commercial products				
			Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry
		SMUTS, etc.—(Continued)															
143	Onion	Urocystis cepulae			x			x					x				
144	Rice	Tilletia horrida			x								x		x		x
145	Rice	Ustilaginoidea virens			x								x				x
146	Rye	Claviceps purpurea				x							x			x	
147	Rye	Urocystis occulta			x								x		x		
148	Sorghum	Sphacelotheca sorghi			x								x				
149	Spinach	Entyloma ellisii			x								x				
150	Timothy	Ustilago striaeformis			x								x				
151	Wheat	Tilletia tritici			x								x		x		x
152	Wheat	Tilletia foetans			x								x		x		x
		SOIL ORGANISMS															
153	Bean	Sclerotium rolfsii					x									x	x
154	Coconut	Thielaviopsis paradoxa					x		x								x
155	Corn	Gibberella saubinettii		x									x				
156	Cotton	Phymatotrichum omnivorum					x										x
157	Eggplant	Pythium debaryanum					x		x							x	
158	Lettuce	Sclerotinia minor					x						x				
159	Mushroom	Mycogone perniciosa						x					x				x
160	Pea	Aphanomyces euteiches					x						x				x
161	Potato	Rhizoctonia solani					x			x					x		
162	Rhubarb	Phytophthora cactorum					x		x				x				
163	Tobacco	Thielavia basicola					x										x
164	Wheat	Helminthosporium sativum			x								x		x		x
165	Wheat	Ophiobolus graminis					x						x				x

TABLE 1.—(*Continued*)

No.	Host	Causal organism	Seeds					Propagating material other than seeds					Commercial products				
			Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry
		BACTERIAL DISEASES															
166	Alfalfa	Aplanobacter insidiosum		x									x				
167	Apple	Bacillus amylovorus			x				x								x
168	Bean	Pseudomonas phaseoli			x								x				
169	Calla Lily	Bacillus aroidae			x					x							x
170	Carrot	Bacillus carotovorus			x								x				
171	Corn	Aplanobacter stewartii			x								x				
172	Gladiolus	Bacterium marginatum					x			x							x
173	Mulberry	Bacterium mori			x				x				x				
174	Pea	Bacterium pisi			x								x				
175	Plum	Pseudomonas pruni			x				x				x				
176	Tomato	Bacillus solanacearum		x				x					x				
		VIRUS TROUBLES															
177	Aster	(Yellows)					x		x				x				
178	Bean	(Mosaic)		x									x				
179	Beet	(Curly Top)					x						x				
180	Celery	(Mosaic)					x						x				
181	Cherry	(Buckskin)					x		x				x				
182	Cucumber	(Mosaic)		x									x				
183	Iris	(Mosaic)					x		x				x				
184	Lily	(Mosaic)					x		x				x				
185	Narcissus	(Mosaic)					x		x				x				

TABLE 1.—(*Continued*)

No.	Host	Causal organism	Seeds					Propagating material other than seeds					Commercial products				
			Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry	Embargo	Detention	Disinfection	Inspection	Unrestricted entry
		VIRUS TROUBLES—(Continued)															
186	Peach	(Phony Peach)					x		x								x
187	Peach	(Yellows)					x		x								x
188	Potato	(Leaf Roll)					x		x				x				
189	Raspberry	(Leaf Curl)					x		x								x
190	Sugar cane	(Mosaic)					x		x				x		x		
191	Tobacco	(Mosaic)					x						x				
192	Tomato	(Streak)					x						x				
193	Wheat	(Rosette)					x						x				x
		DISEASES DUE TO NEMATODES															
194	Beet	Heterodera schachtii					x						x				
195	Chrysanthemum	Aphelenchoides ritzema-bosi					x		x				x				
196	Clematis	Heterodera marioni					x			x							
197	Ferns	Aphelenchoides olesistus					x	x			x		x				
198	Narcissus	Anguillulina dipsaci					x			x			x				
199	Strawberry	Aphelenchoides fragariae					x			x							x
200	Wheat	Tylenchus tritici			x								x				x

INTERSPECIFIC TRANSFER OF A GENE GOVERNING TYPE OF RESPONSE TO TOBACCO-MOSAIC INFECTION[1]

FRANCIS O. HOLMES

(Accepted for publication June 5, 1936)

The hybrid produced by crossing *Nicotiana rustica* L. and *N. paniculata* L. was first studied in 1761 by Kölreuter (7), who showed that repeated pollination of *N. rustica-paniculata* hybrids with pollen from *N. paniculata* resulted in a succession of generations of increasing fertility from the almost completely self-sterile F_1 to a fully self-fertile *N. paniculata*-like form. Kölreuter thus demonstrated that it was possible to obtain what appeared to be the species *N. paniculata* from *N. rustica* by repeated use of pollen alone. Other genetic studies of this hybrid, culminating in a series of papers emphasizing its cytological aspects (2, 8, 9, 10), have appeared since that time. Recently it was shown that specific genes control certain disease responses in the genera Capsicum and Nicotiana (4). A dominant gene *L* (virus-localizing) was found to confer immunity from systemic attack of tobacco-mosaic virus by localizing this virus in necrotic primary lesions in the pepper, *Capsicum frutescens* L. Within this species the gene was transferred from the varieties Tabasco and *minimum* to large-fruited strains by means of intervarietal crosses. Somewhat similar dominant genes were found in certain species of Nicotiana. One of these, in *N. rustica*, was studied in the first generation of the hybrid with *N. paniculata* (4, pp. 996–997). The hybrid *N. paniculata* × *N. rustica* showed a necrotic type of response to infection with tobacco-mosaic virus, resembling in this respect the pollen parent, *N. rustica*. In young plants, infection was followed by systemic necrosis; in older plants, virus was localized in necrotic primary lesions. The hybrid showed no signs of the mottling, with attendant formation of enations, characteristic of the seed parent, *N. paniculata* (5).

This paper is concerned with a succession of backcrosses of the hybrid *Nicotiana paniculata* × *N. rustica* var. *jamaicensis*. The production of this series involved repeated use of *N. paniculata* pollen, as well as retention only of plants that responded to infection with tobacco-mosaic virus by production of necrotic lesions. Subsequent selfing of plants that were heterozygous for the gene determining the necrotic type of response, and that resembled the species *N. paniculata* in appearance, gave rise to homozygous necrotic-type plants.

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EXPERIMENTS

From F_1 plants of constitution *Nicotiana paniculata* × *rustica* a series of 5 generations was produced by application of *N. paniculata* pollen. Plants in the last 3 of these generations proved fertile to their own pollen, and were selfed. The following observations were made on ratios of necrotic-type to mottling-type individuals.

In the first of the backcross generations, 119 plants were grown and inoculated with tobacco-mosaic virus. Although these plants displayed great variability of leaf type and habit, only 2 kinds of response to infection were manifested. These were production of necrotic lesions, as in the F_1 hybrid, and mottling, as in *Nicotiana paniculata*. The ratio of necrotic-type to mottling-type plants was 60 : 59. In this backcross generation, therefore, a 1 : 1 ratio was obtained. Several of the necrotic-type plants were then pollinated with pollen from the mottling-type species, *N. paniculata*. In the second backcross generation, seed for which was obtained with difficulty from 2 plants, not a 1 : 1 but an approximate 2 : 1 ratio between necrotic and mottling types appeared in each of 3 sets. These 2 : 1 ratios were unexpected, and no entirely satisfactory explanation can be given for them with the evidence available at present. The practice of applying pollen of

TABLE 1.—*Ratios of necrotic-type to mottling-type plants in F_1 and 5 backcross generations of Nicotiana paniculata-rustica hybrids. All backcross generations were obtained by pollinating necrotic-type plants of a preceding generation with pollen from N. paniculata*

Generation	Necrotic-type plants	Mottling-type plants	Totals	Observed ratios
F_1 = N. paniculata × rustica	32	0	32 : 0	1 : 0
1st backcross	60	59	60 : 59	1.01 : 0.99
2nd backcross	37	18		
" "	65	34	128 : 68	1.96 : 1.04
" "	26	16		
3rd backcross	100	95		
" "	50	54	204 : 196	1.02 : 0.98
" "	54	47		
4th backcross	57	52		
" "	62	56	188 : 178	1.03 : 0.97
" "	69	70		
5th backcross	26	38		
" "	29	33		
" "	35	32	163 : 158	1.02 : 0.99
" "	34	29		
" "	39	26		

N. paniculata to emasculated flowers of necrotic-type hybrids was continued. In all 3 generations after the second backcross, approximate 1:1 ratios between plants of necrotic and mottling types were obtained. A detailed account of these 5 backcross generations is given in table 1.

Self-fertility comparable to that of the original parents, not observed in earlier hybrid generations, appeared in the third backcross generation. A necrotic-type plant of this generation was selfed. Altogether 288 plants were grown from its seed and tested; the ratio of necrotic-type to mottling-type plants was 210:78, or slightly less than a 3:1 ratio (Table 2, lot 1).

TABLE 2.—*Ratios of necrotic-type to mottling-type plants in 2 selfed generations of Nicotiana paniculata-rustica hybrids*

Lot number	Parent (selfed)	Necrotic-type plants	Mottling-type plants	Totals	Observed ratios
1	Necrotic-type plant of third backcross generation	210	78	210:78	2.92:1.08
2	Necrotic-type plant of lot 1	42	14		
3	" " " " " "	45	16	172:57	3.01:1.00
4	" " " " " "	35	7		
5	" " " " " "	50	20		
6	" " " " " "	67	0	128:0	1:0
7	" " " " " "	61	0		
8	Mottling-type plant of lot 1	0	59	0:157	0:1
9	" " " " " "	0	98		

Eight lots of plants were grown from seeds obtained by selfing 6 necrotic-type and 2 mottling-type plants of this generation. Four of these populations, grown from seeds of necrotic-type parents, showed approximate 3:1 ratios between necrotic-type and mottling-type plants (Table 2, lots 2 to 5). The other 2 populations derived from necrotic-type parents contained only necrotic-type plants, 128 in all (Table 2, lots 6 and 7); these resembled *Nicotiana paniculata* in appearance and were self-fertile. The 2 populations derived from mottling-type parents contained only mottling-type plants, 157 in all (Table 2, lots 8 and 9).

These results indicate that a single dominant gene *N* (necrosis), derived from *Nicotiana rustica*, determines a necrotic type of response to infection with tobacco-mosaic virus, its absence allowing mottling. It is not known just how the *N. rustica* gene *N* finally associated itself with the chromosomes of *N. paniculata*, but it seemed to be able to act in a uniform manner in the twice self-pollinated sets, as it had in backcross populations.

The production of 2 sets containing only necrotic-type individuals resembling *Nicotiana paniculata* in appearance constitutes evidence that the

necrotic-type response of *N. rustica* was incorporated successfully in *N. paniculata*-like segregates in homozygous condition. This was confirmed by reciprocal crosses of one of these individuals with mottling-type *N. paniculata* (*nn*). Only necrotic-type (*Nn*) plants were obtained, 174 with mottling-type *N. paniculata* as pollen parent, and 204 with mottling-type *N. paniculata* as seed parent. It is evident that the gene *N* is capable of being distributed regularly to gametes of both sexes.

Throughout the study of backcross sets only one kind of necrotic-type response to inoculation with tobacco-mosaic virus had been noted. It was not realized that this differed slightly from the response of *Nicotiana rustica*, until certain sets produced by selfing showed two kinds of necrosis. The mode of occurrence of these gave evidence of the presence of a modifying gene.

Five of 8 sets, derived by self-pollination from necrotic-type plants of a fourth backcross set, each showed two kinds of necrotic-type response. One of these was like that shown by all plants in backcross progenies, involving unmodified necrosis. The other was a delayed necrotic-type response associated with peripheral yellowing of lesions. In plants showing the latter response, both primary and secondary lesions were yellowish, with necrosis varying from a trace to a considerable amount; in the systemic phase of the disease this yellowing superficially resembled ordinary mottling. *Nicotiana rustica* responds to infection with necrotic lesions, each of which is surrounded by yellowed tissue. These lesions tend to appear later than those of the necrotic-type backcross plants. In the 5 sets of plants showing the delayed necrotic-type response (Table 3, lots 1–5), the ratio of unmodified necrosis to delayed necrosis was 100 : 33. This 3 : 1 ratio appears to indicate that delay of necrosis, with attendant yellowing of surrounding

TABLE 3.—*Numbers of plants showing 3 types of disease response, unmodified necrosis, delayed necrosis, and mottling, in 8 lots grown from seeds obtained by selfing 8 necrotic-type plants of the fourth backcross generation (see Table 1)*

Lot number	Types of disease response					
	Unmodified necrosis		Delayed necrosis		Mottling	
1	20		7		17	
2	6		1		4	
3	24	100	5	33	27	106
4	24		7		32	
5	26		13		26	
6	28		0		27	
7	43		0		26	
8	31		0		33	
Totals	235				192	

tissue, is dependent on a recessive gene (*d*, delayed necrosis). The recessive gene was evidently not derived from *N. paniculata*, since delayed necrosis was not observed in 5 preceding hybrid generations, representing *N. paniculata* crosses. It must, therefore, have come from *N. rustica*. Furthermore, this gene was not in the chromosome bearing the gene for necrosis (*N*), for the two showed independent segregation. Thus, there was evidence of persistence of a chromosome or chromosome fragment from *N. rustica*, not intentionally selected, as was the carrier of the dominant factor *N*, but probably substituted for a *N. paniculata* chromosome from which it differed by at least one gene. The remaining 3 selfed sets from necrotic-type plants of the fourth backcross set showed no delayed necrotic-type response. Two of them (Table 3, lots 6 and 8) had earlier shared a common parent, a necrotic-type plant of the third backcross generation, with the 5 sets that did show delayed necrosis; the remaining set (Table 3, lot 7) was also closely related.

Simple genetic ratios were observed in all backcross sets, with the possible exception of the second backcross generation as noted; the selfed generations summarized in table 2 also showed customary ratios. In table 3, however, it will be noted that there was a consistent excess of mottling-type plants over the expected 3:1 ratio in all selfed sets derived from plants of the fourth backcross generation. Evidence in hand does not seem sufficient to warrant a conclusion as to the reason for this. Segregation of genes *N* and *D* in these sets seemed regular.

Some evidence was obtained confirming the relationship of *Nicotiana rustica* to other species. It is believed that *N. rustica* (n = 24) originated as an amphidiploid from a cross involving progenitors of *N. undulata* (n = 12) Ruiz and Pavon and of *N. paniculata* (n = 12) (1, pp. 393–395). The response of *N. undulata* to inoculation with tobacco-mosaic virus has not been tested previously. If this explanation of relationships is correct, however, it should resemble the necrotic type displayed in *N. paniculata-rustica* hybrids, unless present-day stocks of *N. undulata* or of *N. paniculata* have lost a dominant gene *N*, now characteristic of *N. rustica*. Seeds of *N. undulata* were obtained through the kindness of Dr. R. E. Clausen, of the University of California. Plants grown from 2 seed lots responded to inoculation with tobacco-mosaic virus by systemic necrosis of the type shown otherwise only by *N. rustica* and the *N. paniculata* hybrids described in this paper. Their response appears to confirm the relationship of the species. Plants from a third lot of seeds of *N. undulata* gave an atypical mottling-type response, which may indicate either a lack of the gene *N*, or another modification of the necrotic-type response similar to that in hybrids of constitution *NNdd* or *Nndd* as described above. The true nature of this response cannot be decided in the absence of genetic data. From the same source 2

other previously untested Nicotiana species were obtained: they were *N. solanifolia* Walp. and *N. raimondii* MacBride, both morphologically distinct from, but genetically closely allied to *N. paniculata;* they both showed a mottling-type response to inoculation with tobacco-mosaic virus, similar to that of *N. paniculata.*

DISCUSSION

A number of hosts of tobacco-mosaic virus respond to infection by simultaneous primary necrosis and localization of virus. This is true, for example, of *Nicotiana glutinosa* L., *N. langsdorffii* Weinm., and *Phaseolus vulgaris* L., which have been used commonly for estimating virus concentration and separating virus strains. The view has recently been expressed by some, that there is a causal relationship between necrosis as a disease symptom and localization of virus. In mature or nearly mature plants of *Nicandra physalodes* (L.) Pers., however, infection with tobacco-mosaic virus is followed by the production of non-necrotic local lesions only. These are marked by starch retention, slight yellowing, or, in old leaves, chlorophyll retention (3, pp. 344–345). Jensen (6) has described yellow-mottling strains of tobacco-mosaic virus that produce only nonnecrotic primary lesions in *Nicotiana tabacum* L. and do not spread systemically. Thus it would appear that localization of virus may occur without necrosis. Systemic necrosis is a comparatively infrequent sequel of infection with tobacco-mosaic virus. It has been described for several species, however, including *N. rustica* (3, pp. 333–337). The present demonstration of a purely necrotic-type systemic disease, with no trace of mottling, in which virus movement is as prompt as in the corresponding mottling-type disease in similar young plants of but slightly different genetic constitution, furnishes an ideal instance of necrosis without virus localization. Since virus localization may occur without necrosis, and necrosis without localization, it appears that there is no necessary causal interrelationship between necrotic-type response and localization of virus.

It is not yet clear what mechanism causes necrotic-type response in the cells of plants receiving the dominant genetic factor *N* (necrosis) to replace the mottling-type response, characteristic of plants of *Nicotiana paniculata* lacking this factor. Evidently, however, it is a mechanism not affected by the numerous chromosomal recombinations involved in the second, third, and fourth hybrid generations described here, in which plant types were found to be extremely varied, but in which only these 2 types of disease response were noted.

Hybrids were made to approach *Nicotiana paniculata* in appearance by means of repeated backcrosses. The dominant gene *N* derived from *N. rustica* was retained as a result of simultaneous selection, and was finally

established in homozygous condition by self pollination of necrotic-type plants. Since the plants are now homozygous for the introduced genetic factor, are self-fertile, and would be identified as *N. paniculata* if found growing wild, the transfer of the *N. rustica* gene is considered an interspecific transfer, and the new strain may for practical purposes be considered a necrotic-type variety of the species *N. paniculata.* It may, of course, differ from the original *N. paniculata* stock in ways not now recognized, for it is probable that not a single gene, but a segment of a chromosome, was carried over from *N. rustica.*

SUMMARY

A necrotic type of response to infection with tobacco-mosaic virus was introduced into the species *Nicotiana paniculata.* This was accomplished by transferring a dominant gene *N* (necrosis) from *N. rustica,* through repeated backcrosses of the hybrid *N. paniculata* × *N. rustica,* using *N. paniculata* pollen, but retaining in each generation only individuals responding to inoculation by production of necrotic lesions. The necrotic-type variety of *N. paniculata* thus produced was self fertile and, in appearance, resembled the ordinary mottling-type *N. paniculata.* In its response to infection, however, it was essentially like *N. rustica,* dying from systemic necrosis if infected when young, localizing virus if infected when old.

A dominant gene *D* (unmodified necrosis), not found in *Nicotiana rustica,* was observed in the newly derived necrotic-type *N. paniculata* plants. It was found to segregate independently with respect to the gene *N* (necrosis). In the presence of *N,* the gene *D* allowed necrotic primary lesions to appear promptly, and prevented extensive yellowing of surrounding tissue.

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LITERATURE CITED

1. GOODSPEED, T. H. Nicotiana phylesis in the light of chromosome number, morphology, and behavior. California Univ. Pubs., Bot. 17: 369–398. 1934.
2. ————, R. E. CLAUSEN, and R. H. CHIPMAN. Interspecific hybridization in Nicotiana. IV. Some cytological features of the *paniculata-rustica* hybrid and its derivatives. California Univ. Pubs., Bot. 11: 103–115. 1926.
3. HOLMES, F. O. Symptoms of tobacco mosaic disease. Contrib. Boyce Thompson Inst. 4: 323–357. 1932.
4. ————. Inheritance of ability to localize tobacco-mosaic virus. Phytopath. 24: 984–1002. 1934.
5. JENSEN, J. H. Leaf enations resulting from tobacco-mosaic infection in certain species of Nicotiana L. Contrib. Boyce Thompson Inst. 5: 129–142. 1933.

6. JENSEN, J. H. Studies on the origin of yellow-mosaic viruses. Phytopath. **26**: 266–277. 1936.

7. KÖLREUTER, J. G. Ostwald's Klassiker der exakten Wissenschaften, nr. 41. Kölreuter's Vorläufige Nachricht von einigen das Geschlecht der Pflanzen betreffenden Versuchen und Beobachtungen, nebst Fortsetzungen 1, 2, und 3 (1761–1766). 266 p. Hrsg. von W. Pfeffer. W. Engelmann, Leipzig. 1893.

8. LAMMERTS, W. Interspecific hybridization in Nicotiana. IX. Further studies of the cytology of the backcross progenies of the *paniculata-rustica* hybrid. Genetics **14**: 286–304. 1929.

9. ————. Interspecific hybridization in Nicotiana. XII. The amphidiploid *rustica-paniculata* hybrid; its origin and cytogenetic behavior. Genetics **16**: 191–211. 1931.

10. SINGLETON, W. R. Cytogenetic behavior of fertile tetraploid hybrids of *Nicotiana rustica* and *Nicotiana paniculata*. Genetics **17**: 510–544. 1932.

BLACK STEM OF ALFALFA IN IDAHO[1]

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INTRODUCTION

A disease of alfalfa, which causes a distinct blackening of stems and petioles and a spotting of the leaves, has been especially prevalent in Idaho during the last 2 years. In previous years various collections and observations of discolored alfalfa stems have been made. The cause usually was attributed to *Phytomonas medicaginis* Sack., the organism causing bacterial stem blight. Careful culture work, involving numerous collections during 1934 and 1935, revealed the fact that a fungus organism is, in the majority of cases, associated with dark brown or black lesions so often found on alfalfa stems in Idaho. Valleau and Fergus (5), and later Johnson and Valleau (1), reported a fungus disease on alfalfa in Kentucky, which they have named black stem. They state that black stem is caused by *Phoma medicaginis* Malbr. and Roum. From their description of the disease and the causal organism, it appears that the disease with which we have been working in Idaho is the same as the one occurring in Kentucky.

Richards (2) recently reported upon resistance of certain alfalfa varieties to stem blight in Utah. Although he intimates that the disease under discussion is the bacterial stem blight (*Phytomonas medicaginis*) he states in a footnote that a species of Phoma has been isolated with considerable uniformity. Richards did not describe the symptoms of the disease in question.

OCCURRENCE OF BLACK STEM IN IDAHO

Following the mild winter of 1933–1934, an outbreak of stem rot of alfalfa and clover, caused by *Sclerotinia trifoliorum* Eriks., occurred in certain sections of northern Idaho. The stems of stem-rot diseased plants submitted for examination were badly blackened in many cases, and cultures from these lesions indicated the presence of a species of Phoma. A careful study has been made of this type of injury upon alfalfa, and the black-stem disease has since been collected on alfalfa from 12 different, widely distributed counties in Idaho. It appears that the disease is generally distributed throughout the State. The bacterial stem blight described by Sackett (4) is also widely distributed in the State, as evidenced by observations made

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during the last 20 years. The early symptoms of the bacterial disease differ sufficiently from black stem to make it easily possible to differentiate between them. Later in the progress of the bacterial disease, lesions turn darker and more closely resemble the lesions of the black-stem disease.

The damage caused by black stem is much more severe on the first cutting of alfalfa. As a matter of fact, it is rather difficult to find the disease on later cuttings. It is to be found throughout the season, however, on stubble and on plants that have not been cut for hay. Although the percentage of loss and reduction of stand from this disease is difficult to estimate, observations would indicate that this loss has been considerable where the disease has been severe.

DESCRIPTION OF THE DISEASE

The characteristic symptoms of the disease consist of dark brown to black areas on the stems and petioles, and black spots on the leaves (Fig. 1, A). Under field conditions in Idaho, the blackened areas are largely confined to the stems and petioles, with few leaf spots, while under greenhouse conditions, the leaf spots are more conspicuous. The stem lesions originate as small black spots, which rapidly enlarge, coalesce, and form larger areas. These areas may extend for several inches along the stem and may entirely girdle it. In the early stages of the disease the infection is confined mainly to the surface of the stem, but later may penetrate to the interior. Leaves that are severely spotted become etiolated and finally drop off. In heavy stands of alfalfa, young shoots may be injured and die, or the tips may be killed back. This is especially true under nonirrigated conditions when the spring season is long, wet, and cool, and also in irrigated regions where conditions are favorable for rank growth and lodging of plants. Under such conditions stems may be blackened their entire length and become badly distorted and dwarfed. On dead stems and leaves, fruiting bodies of the organism are present in abundance (Fig. 1, C).

THE CAUSAL ORGANISM

The causal organism is easily isolated from blackened stems and petioles and from the spots on the leaves. It also is possible to isolate the same organism from the fruiting bodies on the dead tissue. The organism was isolated in March, 1935, from black areas on dead alfalfa stems collected and kept in herbarium folders since 1925.

A culture of *Phoma medicaginis*, obtained from W. D. Valleau in Kentucky, proved to be identical with the organism isolated from alfalfa in Idaho. Several cultures, isolated from alfalfa collected in various parts of Idaho, and the one from Kentucky were compared on potato dextrose, cornmeal, prune, and alfalfa-extract agar, and on sweet-clover stems, when

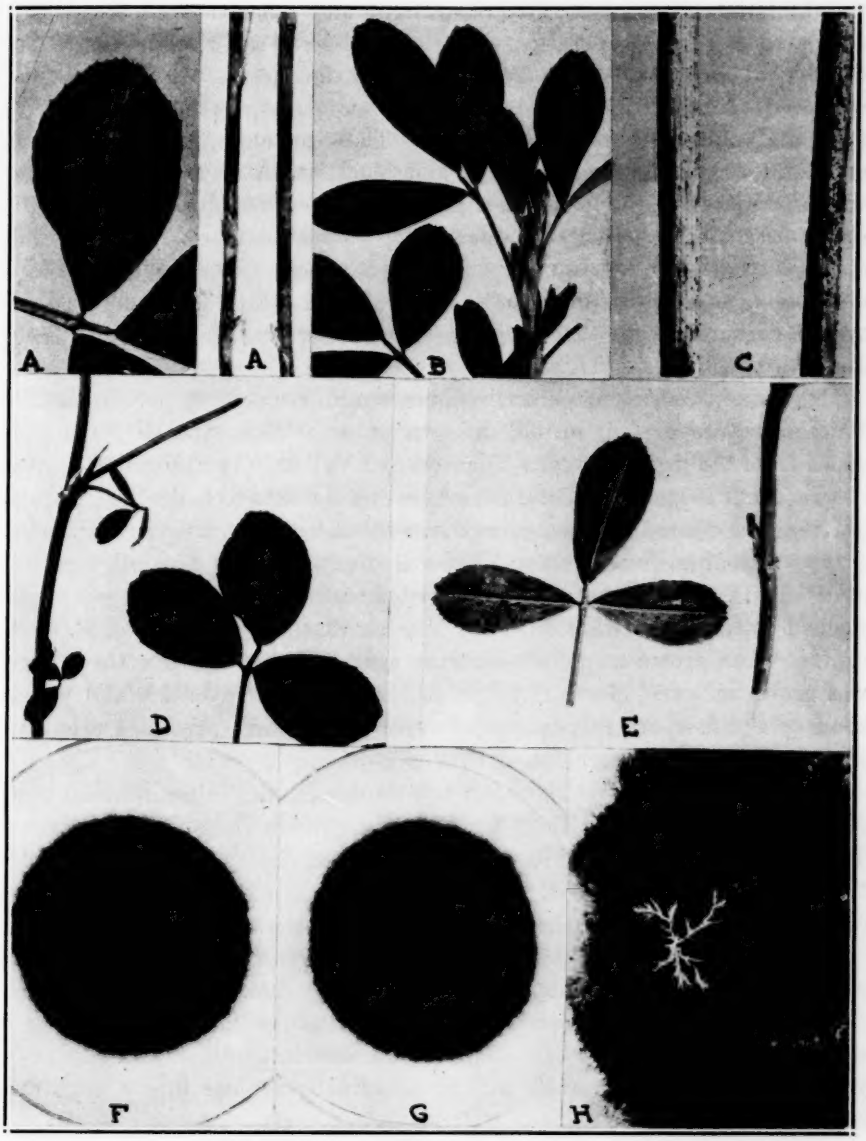

FIG. 1. A. Symptoms of black stem on alfalfa leaves and stems from the field. B. Healthy alfalfa. C. Fruiting bodies overwintering on alfalfa stems. D. Stems and leaves from alfalfa plant, inoculated with *Phoma medicaginis*. E. Stems and leaves from plant, inoculated with *Pleospora rehmiana*. F. Culture *P. medicaginis*, reisolated from inoculation (D). G. Culture of *P. medicaginis*, reisolated from inoculation with *Pleospora rehmiana* (E). H. Typical crystals that appear in cultures of *P. medicaginis*.

grown under different conditions of light and temperature. They were compared at room temperature under normal light and darkness, at 0°–5° C. in total darkness, and at 26° C. in total darkness. All these cultures appeared identical in reaction to media and in growth characteristics. Pycnidia appeared in four to six days. These pycnidia, at first, were soft and light brown, but became hard and black, when mature. The pycnospores escape from the ostiole in a gelatinous mass or ribbon, and are cylindrical or oblong, hyaline and unicellular. However, many 2-celled pycnospores have been found in older cultures. Crystals appeared in all potato-dextrose agar cultures in 4 to 10 days (Fig. 1, H). These crystals are typical formations of *P. medicaginis* on potato agar and also were noted by Johnson and Valleau (1).

It was noticed, on close microscopic examination, that perithecia of a Pleospora were present on old dead tissue of alfalfa, especially the dead stems from the previous year. Johnson and Valleau (1) also report having observed a Pleospora fruiting on overwintering stems of alfalfa. Similar perithecia appeared on sweet-clover stems that had been used in the laboratory as a medium for culturing *Phoma medicaginis,* after 3 month's growth at 0°–5° C. Single-spore isolations of ascospores from these perithecia yielded cultures somewhat different from single spore isolations of *P. medicaginis* when grown on potato-dextrose agar. However, when the culture was grown on sweet-clover stems, it produced typical pycnidia and pycnospores. Single-spore reisolations of these pycnospores produced a culture identical to that of the original *P. medicaginis.*

In brief, then, the pycnidia and pycnospores found in nature were comparable in every way to those produced in culture from tissue isolations. The ascigerous stage found in nature was the same as that developed in cultures of the imperfect form, and ascospore cultures from nature and from cultural media produced the imperfect stage of the fungus.

It appears, then, that the Pleospora is the perfect form of *Phoma medicaginis,* and it has been identified as *Pleospora rehmiana* (Staritz) Sacc. (3, p. 1033). The perithecia are immersed to somewhat free; the ascus is elongate-clavate, 115–130 × 28–35 μ; the ascospores are in 2 to many rows, yellowish brown, transversely and longitudinally septate into a muriform condition, 30–35 × 15–18 μ.

PATHOGENICITY

Plants were successfully inoculated in the greenhouse and laboratory by different methods. In some cases, inoculation was made by transferring the organism on agar directly from cultures to the healthy tissue. In other cases, plants were sprayed with a suspension of pycnospores of the causal

organism. Infection in all cases was found to occur readily, even when the plants were not wounded when inoculated.

Alfalfa plants, brought into the greenhouse from the field, Grimm and common alfalfa, and yellow-blossom sweet clover plants that had been grown from seed in the greenhouse were successfully inoculated with pure cultures of the organism. Typical symptoms of black stem were observed 2 to 4 weeks after inoculation. Black lesions appeared on stems and petioles and numerous black spots developed on the leaves (Fig. 1, D). The causal organism, *Phoma medicaginis,* was easily reisolated in all cases (Fig. 1, F).

Common and Grimm varieties of alfalfa and yellow-blossom sweet clover plants also were inoculated in the greenhouse with cultures of *Pleospora rehmiana.* Symptoms were produced that were identical to those produced by *Phoma medicaginis* (Fig. 1, E). In this case, also, the organsim that was reisolated was *Phoma medicaginis* (Fig. 1, G).

Single-spore cultures of *Pleospora rehmiana* and single-spore cultures of *Phoma medicaginis* produced the same symptoms on alfalfa and sweet clover stems and leaves in moist chambers in the laboratory. Spotted areas on stems, petioles, and leaves appeared in 2 to 5 days, and pycnospores were produced in 5 to 8 days after inoculation.

SUMMARY

The black-stem disease of alfalfa in Idaho appears to be the same disease as that reported from Kentucky. The causal organism is identical with *Phoma medicaginis,* which has been identified by Valleau as causing black stem of alfalfa in Kentucky. *Phoma medicaginis,* when grown on sweet clover stems, produced an ascigerous stage corresponding to *Pleospora rehmiana,* which was found also on dead alfalfa stems from the field. Pycnidia of *Phoma medicaginis* were produced on alfalfa and sweet clover in moist chambers after inoculation with a culture of *Pleospora rehmiana.* Typical black-stem symptoms were produced also on alfalfa and sweet clover in the greenhouse when inoculated with *Pleospora rehmiana;* when reisolated, it proved to be identical with the original culture of *Phoma medicaginis.*

It is demonstrated that *Pleospora rehmiana* is the perfect stage of *Phoma medicaginis,* the ascospores of which also function as inoculum for black stem in the alfalfa fields in the spring.

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LITERATURE CITED

1. JOHNSON, E. M. and W. D. VALLEAU. Black-stem of alfalfa, red clover, and sweet clover. Kentucky Agr. Expt. Sta. Bull. 339. 1933.
2. RICHARDS, B. L. Reaction of alfalfa varieties to stem blight. Phytopath. 24: 824–827. 1934.
3. SACCARDO. Sylloge fungorum. V. 24, Sec. 2. Sumptibus Coheredum Saccardo, Abellini, 1928.
4. SACKETT, W. G. A bacterial disease of alfalfa. Colo. Agr. Expt. Sta. Bull. 158. 1910.
5. VALLEAU, W. D. and E. N. FERGUS. Black stem of alfalfa, sweet clover, and red clover. Phytopath. 19: 507–509. 1929.

THE DISSEMINATION OF SEPTORIA ACICOLA AND THE EFFECT OF GRASS FIRES ON IT IN PINE NEEDLES[1]

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INTRODUCTION

Septoria acicola (Thüm.) Sacc. causes a serious needle blight of longleaf pine seedlings. Investigations of the effectiveness of periodic grass fires in controlling the disease in natural reproduction[2] have led those working with it to believe that its dissemination is chiefly by spores carried in rain drops, splashing from infected plants. The present investigations were made in Washington Parish, Louisiana, in 1933, to get more precise information on the dissemination of the fungus and the effect of grass fires on it in needle tissue.

THE DISSEMINATION OF SPORES

The general infection of the 1932 needles to a height of 6 feet by *Septoria acicola* in the Bogalusa area of Washington Parish and the presence of a noticeable amount of the bar-spot type of infection (1 to 2 per cent of the leaf length dead on some trees)[3] on mature trees at a height of 75 feet indicates that, under certain conditions, Septoria can be appreciably wind-disseminated. There was indirect evidence, however, that during the first half of 1933 wind dissemination was not abundant. Seedlings over 2 feet high did not develop many spots on the 1933 foliage, although on smaller seedlings infection was of epidemic rating as usual.

There also exists the possibility of dissemination by insects, especially by mites and a large grasshopper common during the winter months and early spring. However, no spores were found by direct microscopic observation or in washings of insects. The culture method would be better for such studies, but Septoria does not lend itself to this method, and direct observation of spores on opaque objects is not always easy or certain. However, if insects commonly carried large numbers of spores, some probably would have been found.

[1] The investigations here reported were made by the writer while employed as Field Aid by the Division of Forest Pathology, Bureau of Plant Industry, in cooperation with the Southern Forest Experiment Station, U. S. Forest Service, New Orleans, La. Much assistance was given by Mr. P. V. Siggers of the Division of Forest Pathology.

[2] Siggers, P. V. Observations on the influence of fire on the brown-spot needle blight of longleaf pine seedlings. Jour. Forestry 32: 556–562. 1934.

[3] The bar-spot type of infection is indicative of resistance. On such spots the fungus seldom fruits or develops extensively. (Verrall, A. F. The resistance of saplings and certain seedlings of *Pinus palustris* to *Septoria acicola*. Phytopath. 24: 1262–1264. 1934).

Direct evidence tended to show that most of the spores are disseminated by splattering rain. From the middle of January until June 30, 1933, trap slides were kept in an epidemic area of dense seedling stocking, and examined daily. Standard microscope slides were smeared with vaseline as an adhesive until June, when the temperature became high enough to liquefy vaseline. A thin layer of "Tree Tanglefoot" was then used. Slides were placed horizontally and vertically sufficiently near diseased needles to be splattered by rain drops hitting the leaves and at heights of 1½ and 3 feet, respectively, above seedlings. During the 6 months, numerous spores were caught on 3 occasions: January 24–25, May 1–2, and June 10–11. On these occasions and on at least one day previous, it rained, so that there were at least 29 hours with relative humidities above 80 per cent each time spores were caught. Slides placed near enough to diseased seedlings to be splattered by rain drops from infected seedlings collected 100–400 spores per slide, and on May 1 and 2 when heavy winds accompanied the rain 50 spores per slide were caught at 3 feet above seedlings. During the shorter rains on other days a few spores were caught under diseased plants, but, between rains, only 3 solitary spores were caught. The duration of the wet period in the usual summer shower often is not long enough for spore discharge, the fruiting bodies presumably drying before spores are discharged. The temperature probably is too low for sporulation during many winter rains; the January 24–25 discharge was, however, an exception, the weather at that time being warm (53–80° F. on January 23 to 25).

The theory that spore dissemination occurs mainly through the splashing of rain also was tested by dividing a 10×10 foot plot into 2 sections. On one the infected 1932 foliage was left on the plants; on the other, all 1932 foliage was removed and the section screened on the sides with a double thickness of cotton cloth 15 inches high. Within 5 to 10 feet of the plot were numerous seedlings with heavily infected 1932 foliage extending more than 15 inches above the ground so that it is likely that any wind-blown spores from these could reach the plants within the screened plot. All the seedlings over 12 inches high were removed from a 5 foot border around the plot to prevent splattering over the screen. The new foliage on the plants in the nonscreened part of the plot were subject to inoculation by spores from infected 1932 foliage, both within and surrounding the plot, as well as by any wind-disseminated spores. At the time of establishment in March, 1933, some of the small seedlings had leaf buds opening, but the buds of most of the plants had not yet opened. On May 20, there was a noticeable difference in the degree of infection on the two plots, the new foliage on the plants within the screen being fairly free (3.9 per cent of the leaf length dead), while those without were heavily infected (14.4 per cent of the leaf length dead). On June 23 the amount of the 1933 leaf length dead was 7.6 and

25.0 per cent, respectively. This infection appeared to be due chiefly to the spore shower of May 1–2.

If the spores of Septoria were commonly wind-disseminated one would not expect much decrease in the degree of infection on fire lanes 100 feet wide in an epidemic area as compared with the adjacent unburned areas. Fire lanes are made in the fall by burning strips about 100 feet wide at strategic places across the area to be protected. The grass and the needles of the previous year on pine seedlings are burned. The lanes are flanked by a narrow plowed zone. In the Bogalusa area, however, there was a marked reduction in the infection on fire lanes (Table 1). Each figure is based on 50 seedlings. Only the 1933 needles were included in the estimate.

TABLE 1.—*Percentage of leaf length dead on June 20, 1933, on adjacent burned and unburned areas in Washington Parish, Louisiana*

Lane no.	Unburned to right of lane	Right 5 ft. of lane	Center of lane	Left 5 ft of lane	Unburned to left of lane
1	15.3	3.6	2.1	4.2	15.2
2	15.4	4.8	1.6	2.9	15.1

A 15-foot road borders the right edge of lane 1 and about 5 feet of plowed soil on the opposite side and both edges of lane 2. Similar reductions in the degree of infection have been observed on other fire lanes.

The behavior of the disease on burned areas[4] suggests that there is but little wind dissemination. The fire, if severe, destroys most of the old infected leaves, and the wind brings in a few spores that cause a low degree of infection the first season. During the spring of the second year, with the rapid extension of the fungus from the points of inoculation, enough spores are produced for a general inoculation of the new foliage through splattering of rain, resulting in the usual great increase in the amount of infection the second season after a burn in areas of severe seedling infection, even though it takes 5 or 6 growing seasons after a burn for infection to reach a maximum.[4]

THE EFFECT OF FIRE OF SEPTORIA IN PINE NEEDLES

The severity of grass fires varies greatly, depending on a number of factors; and the amount of longleaf seedling needles killed by the fire likewise varies from a browning of the tips to complete destruction. A number of moist chamber and agar cultures were made of leaves to determine what fire intensities sterilize brown-spotted needles. The 6 fires examined occurred in January, February, March, and April, 1933, and in no case did

[4] Siggers, P. V., *loc. cit.*

Septoria fruit, nor could isolations be obtained from leaf tissue killed by fire. The killed tissue used varied from charred to that uncharred and apparently killed by temperature near the thermal death point of leaf tissue. Plantings were made within 2 days of the fire and again 1 to 3 weeks later. In all, 96 plantings were made on agar and about 500 moist chamber needles were examined without finding living Septoria. Plantings, just after a fire, were all sterile; those after a week yielded mostly Pestalozzia.

Fruiting was abundant on unburned leaves in the same area at the time of these tests and cultures were easily secured from them. Fruiting also was abundant on the green bases of needles with fire-killed tips up to the end of the live portion and also for a period up to 2 weeks on the dead areas gradually dying back from the fire-killed tips. Pestalozzia, Lophodermium, and other fungi soon take over the fire-killed tips and, as the green bases die, quickly exclude Septoria.

SUMMARY

The indirect and direct evidence available indicates that the spores of *Septoria acicola* are wind-disseminated to a slight extent, and that most dissemination is local by spores carried in rain drops splattering from diseased plants. It appears that for abundant spore discharge 2 or more warm rainy days are needed. Ordinary summer showers are not of sufficient duration for much spore discharge and during winter rains the temperature appears to be too low for spore discharge.

Temperatures that kill leaf tissue also kill the Septoria in that tissue.

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A DISPOROUS GNOMONIA ON PECAN

J. B. Demaree and J. R. Cole

(Accepted for publication December 7, 1935)

In 1917 Matz[1] reported the occurrence in Florida of a Gnomonia on pecan leaves in the ascus of which only two spores were produced. The following year he again referred to the disease as pecan leaf-blotch caused by *Gnomonia* sp. Apparently, there is no record of later collections of the fungus until the writers collected it in the summer of 1928 in a pecan orchard near Monticello, Florida. The following year it was found again in the same orchard, and also at Thomasville, Putney, and Leesburg, Georgia. Since then it has frequently been observed in various orchards in southern Georgia.

Specimens of the fungus collected by Matz on pecan leaves in 1916 were preserved by the Office of Pathological Collections, United States Department of Agriculture, and are listed as *Gnomonia* sp. Those specimens have been examined by the writers, and, from all appearances, the fungus is the same as the one here described.

THE DISEASE

About the middle of June lesions first appear on the leaves as small, inconspicuous brown spots, showing on both sides of the leaflet, with no certain diagnostic features. The spots enlarge rather rapidly and frequently attain a diameter of 0.75 inch. Normally the necrotic areas are circular, but their spread is often so limited by the midrib and larger leaf veins that they become long and narrow. The spots frequently occupy 2 and perhaps 3 adjacent spaces formed by the leaf veins. (Fig. 1.)

The spots vary from a light brown or tan to almost black. The shade of tan is associated with the production of perithecia, which appear in mid or late July. Were it not for the perithecial beaks and the characteristic delimitation of the spots by the vascular tissues, the disease might be confused with lesions caused by *Cercospora fusca* Rand. The number of initial infections on leaflets is commonly not large, varying usually from 1 to 5, but the spots became numerous after secondary infections take place.

THE FUNGUS

No conidial stage has been found on either affected leaflets or artificial cultures. The surfaces of the lesions on both sides of the leaflets are devoid of hyphae, except that a scant amount of white mycelium may be present along the advancing edge of the young spots. These aerial hyphae, however,

[1] Matz, J. An undescribed Gnomonia on pecan leaves. Florida Agr. Expt. Sta. Ann. Rept. 1917: 89R–94R. 1918.

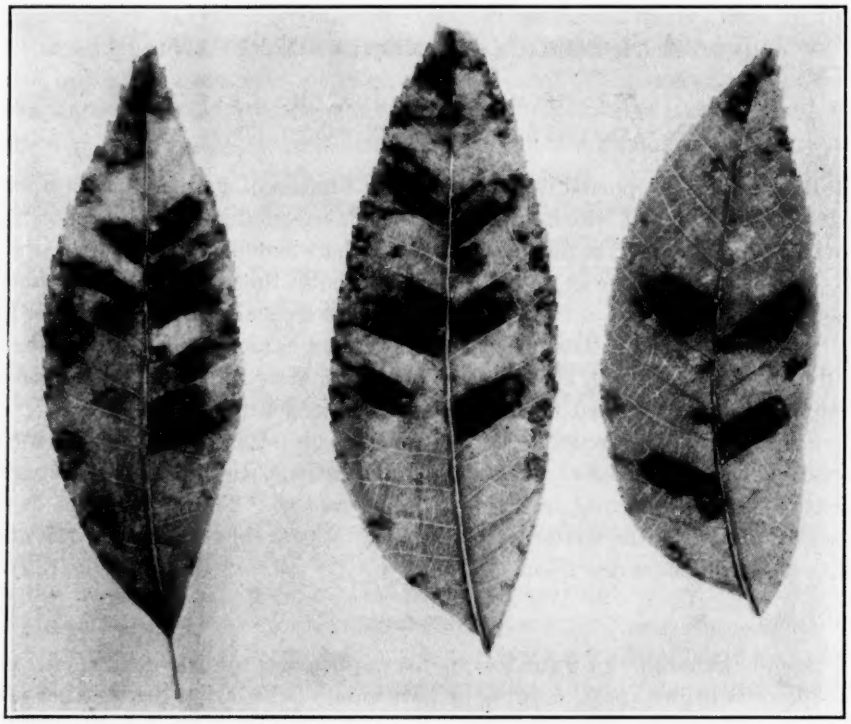

FIG. 1. Pecan leaflets spotted with *Gnomonia dispora* n. sp.

are found only during extended periods of frequent rains and overcast skies, or when affected leaflets are kept in a moist chamber.

The perithecia form and mature on the living leaves in late summer. Mature ascospores have been found as early as mid-July, only 4 or 5 weeks after the first evidence of infections.

The perithecia are buried within the spongy mesophyll of the leaf tissues and not infrequently cause the upper epidermis of the leaf to bulge and even break. The ostioles or beaks are black and frequently curved; most of them penetrate the lower or ventral side of the leaf surface. Only rarely do they break through the upper epidermis.

The perithecia, exclusive of the beak, are spherical to ellipsoidal with thin walls of only 2 to 4 cells thickness. The walls of the beak are thicker and composed of smaller cells than those of the main body of the perithecium.

Two spores commonly are formed in each ascus, and occasionally there is but 1, and less often 3 or 4. The ascus wall is hyaline, very thin, and frequently seems to be distended by the spores. (Fig. 2.) The base of the ascus is drawn out into a needle-shape appendage, which, when detached,

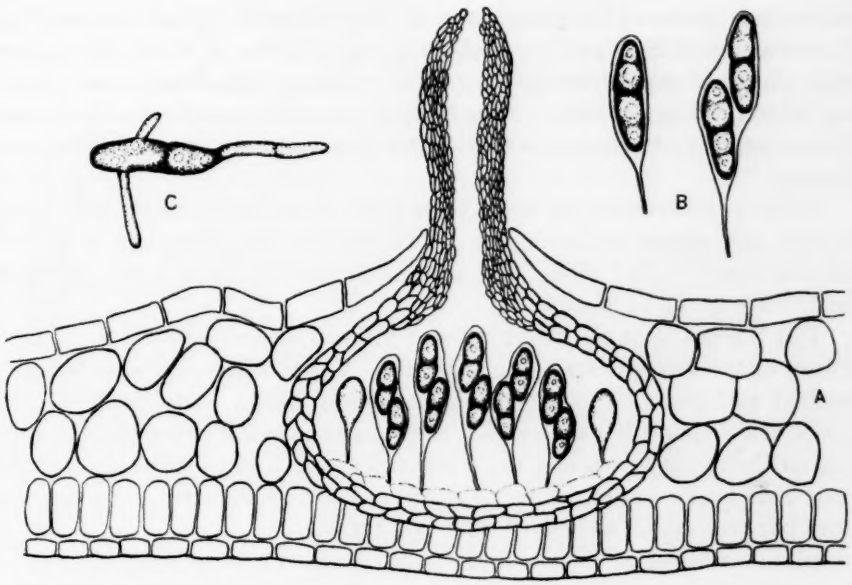

FIG. 2. *Gnomonia dispora* n. sp. *A*. Perithecium (×500); *B*. Asci (×625); C. Germinating spore (×650).

often is bent downward in the form of a hook. The shape of the asci varies and seems to be determined by the arrangement and number of the spores. They are cylindrical when a single spore is present or when two spores lie in a row; ovoid to obovate when the spores are side by side; and irregularly spindle-shape when the spores are imbricate.

The spores are guttulate, 1-septate, hyaline, slightly constricted at the septum, and vary considerably in size. When a single spore is formed in an ascus, it is much larger than when 2, 3, or 4 are present, but there may be 1 large and 1 small spore in the same ascus. When 3 spores are present in a single ascus, 2 may be about normal size and 1 smaller, or there may be 2 small spores and 1 large one. A very few 4-spore asci have been observed and they contained only small spores. The cells of the spores are of unequal size; but this character is also variable, as the difference may be only slight. When an ascus contains 2 spores, the smaller cells usually are situated at opposite ends of the ascus. The germ tubes may project from the sides or from either end of the spores.

Apparently, Matz did not consider the fungus of sufficient importance to furnish measurement, and did not propose a specific name. The fungus is found quite frequently in southern Georgia and northern Florida on pecan and on other species of Hicoria. The disease is considered only a minor one on pecans, and is often associated with rosette, a zinc-deficiency disease.

Since other species of the genus Gnomonia have been described as occurring on overwintered leaves of the hickory group of trees, 2 being the perfect stage of forms causing serious disease of pecans, it has been found inconvenient to refer to this 2-spore form in literature as *Gnomonia* sp. To clarify the matter, it is proposed here to give this 2-spore form the name *Gnomonia dispora*.

As far as the writers can learn, there has been no disporous Gnomonia, or in fact, any disporous ascomycete, with the possible exception of *Microsphaeria alni* (Wallr.) Wint., described as causing lesions on green leaves of the Hicoria group of plants.

This Gnomonia on the pecan differs widely in several respects from other species of the genus described on Hicoria. These differences may be summarized as follows: This Gnomonia commonly has 2, occasionally 1, and rarely 3 or 4 spores in each ascus. The fungus causes a characteristic spotting on the living leaves and the perithecia develop and mature during the latter half of the season, while the leaves are still on the trees. No conidial stage has been found associated with this fungus.

All other forms of Gnomonia described on Hicora have been reported to have 8 spores in each ascus, and their perithecia develop only on overwintered leaves. The writers believe that the disporous form is an undescribed species with the following description: **Gnomonia dispora,** n. sp.

Perithecia scattered, submerged, subglobose to flattened, submembranaceous, dark-brown to black, 80–150 μ by 100–160 μ, neck long, protruding, black. Asci cylindrical, irregular, thin-wall, 2-spore, 55–77 μ by 14–17 μ. Paraphyses none. Spores uniseptate, slightly constricted at septum, spindle-shape to cylindrical, hyaline, guttulate, 24–51 μ by 5–10½ μ.

Habitat: On living leaves of *Hicoria pecan* Britt., Monticello, Fla.; Thomasville, DeWitt, and Leesburg, Ga. Type specimens have been deposited in the Herbarium of the Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

Pure cultures of *Gnomonia dispora* were obtained by first macerating the perithecia, then picking the ascospores out with a needle with the aid of the binocular microscope and placing them in sterile Petri dishes of cornmeal and lima-bean agar. Germination took place within 24 hours at room temperature. Germinated spores were then transferred to Petri dishes and tubes of agar. The fungus was grown on a variety of artificial media, but made the best growth on a medium of cornmeal and potato agar. It grew rapidly on this substratum and attained a colony diameter of approximately 5 cm. in 30 days. Perithecia formed in abundance in about 20-day-old cultures of cornmeal-potato agar when kept near the optimum temperature of 24° to 26.5° C. The perithecia, asci, and ascospores developed in cultures were in no way different from those produced on the host under natural conditions. No conidia were observed in any of the cultures on artificial media.

Cornmeal-agar cultures with a pH range of 3.8 to 9.5 were kept at the optimum temperature over a period of 30 days. These studies also indicated that the organism thrives best on an acid or slightly alkaline medium. The maximum colony growth, 5 cm. in diameter, was made on media ranging on the pH scale from 5.0 to 6.5. On more alkaline agar the fungus grew very slowly.

SUMMARY

A spotting of pecan foliage caused by a fungus, previously reported by Matz, is described. Matz did not suggest a specific name, but referred to it as *Gnomonia* sp., and it is here described as *Gnomonia dispora* n. sp.

Perithecia form and mature in necrotic areas on the living leaves in late summer.

Commonly 2 spores, occasionally 1, and less often 3 or 4, are formed within a single ascus. As a rule, when there is only a single spore in an ascus it is abnormally large; when there are 2 per ascus they usually are about coequal; and when there are 3 one of them may be abnormally large and the other 2 small, or there may be 2 average-size spores and 1 much smaller.

No conidial stage has yet been found.

Normal perithecia, asci, and spores were grown on a mixture of cornmeal and potato agar in about 20 days at the optimum temperature of 24° to 26° C.

ALBANY, GEORGIA

CONCENTRATION OF AMMONIA NECESSARY IN A LOW-LIME PHASE OF HOUSTON CLAY SOIL TO KILL THE COTTON ROOT-ROT FUNGUS, PHYMATOTRICHUM OMNIVORUM

D. C. Neal and Emerson R. Collins

(Accepted for publication December 6, 1935)

In previous experiments[1, 2, 3] it was shown that ammonia in relatively dilute concentrations is toxic to the cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar. In additional laboratory tests, conducted in 1934, the mycelium in sectional agar blocks was killed with ammonia water after 24 hours with a concentration of 50 p.p.m. NH_3; and the sclerotia from 2-year-old soil cultures in 1 hour with a concentration of 300 p.p.m. Although the above data are of interest from the standpoint of probable utilization of ammonia and ammonium compounds for treatment of areas infested with root rot, it is essential to know the approximate concentration of ammonia in the soil necessary to suppress development of the fungus before applications can be made in the field that may give promise of reducing the disease.

When ammonia is applied to types of soil such as constitute the principal root-rot areas of the Texas black-land belt, one is immediately confronted with the problems of base exchange, adsorption, and nitrification, all of which affect to a greater or less degree the concentration of free ammonia that may be obtained from specific applications. In order to obtain some idea of the concentration necessary in a low-lime Houston clay soil to kill both cotton root-rot mycelia and sclerotia, laboratory experiments involving soil treatments with ammonia water and subsequent inoculations with the fungus were conducted in the spring of 1935. Seven hundred and fifty grams of soil (652 g., dry weight) was used for each concentration of ammonia tested, and the range of concentrations varied from 300 to 1325 p.p.m. in increments of 75 p.p.m. In the 300 to 975 p.p.m. range, the moisture content of cultures was adjusted to 27 per cent dry basis, while in the higher ranges, 1025 to 1325 p.p.m., it was 30 per cent. In obtaining the different concentrations of ammonia water used in the soil treatments, the

[1] Neal, D. C., R. E. Wester, and K. C. Gunn. Treatment of cotton root-rot with ammonia. Science, new ser., 75: 139–140. 1932.

[2] Neal, D. C., R. E. Wester, and K. C. Gunn. Growth of the cotton root-rot fungus in synthetic media and the toxic effect of ammonia on the fungus. Jour. Agr. Res. [U. S.] 47: 107–118. 1933.

[3] Neal, D. C. Recent investigations of cotton root rot in Texas. (Abst.) Phytopath. 24: 838. 1934.

amount of N as NH_3 required for each soil concentration in parts per million was calculated and the equivalent supplied from a 0.5801 N solution of ammonium hydroxide, previously standardized against standard acid (H_2SO_4 with methyl red indicator). The required amount of ammonium hydroxide was diluted with water, in the proper proportions, so that the ammonium hydroxide and water brought the soil to the moisture content given for each series. Each concentration of the ammonia water was evenly distributed throughout each soil culture by mixing with spatulas on a mixing board. After treatment, the soil was then transferred immediately to quart Mason jars equipped with modified lids[4] to facilitate handling incident to making inoculations. The jars were then plugged with cotton to prevent escape of the ammonia, and 48 hours was allowed to elapse after the soil was treated before making inoculations. Inoculations were made by inserting from 20 to 30 root-rot sclerotia[5] in the center of each jar, and later firmly pressing the soil around them. Sectional agar blocks of the fungus from fresh cultures also were inserted in the soil of each jar. Jars containing untreated soil but adjusted to the same moisture content (27 and 30 per cent, respectively, dry basis) also were inoculated as controls.

Examinations for evidence of growth of the fungus were made 13 days after inoculation in the 300 to 975 p.p.m. range of concentrations and after 26 days in the 1025–1325 range. Good growth occurred in the cultures with concentrations of 300 to 700 p.p.m.; scant growth at 975 p.p.m.; and no growth at 1025 p.p.m. or in the other higher concentrations employed. After intervals of 26 and 42 days respectively from the dates of treatment, some of the cultures were analyzed for ammonia, nitrate nitrogen, and moisture content, and these results are shown in table 1.

The results show that when ammonia is added to a soil of the above type in the form of ammonia water, its conversion to nitrate is fairly rapid, especially when initial applications are in excess of 825 p.p.m. At these concentrations approximately 30 per cent of the ammonia was nitrified after the 26-day interval. The percentage of conversion also, as was to the expected, was greater in the higher concentrations and with the longer interval preceding analysis. In view of nitrification, the concentration of free ammonia obtained at the time of analysis of the cultures in which the fungus did not grow cannot be considered as the effective concentration for kill. Doubtless it is higher, but further work, using much shorter periods of incubation of the fungus after treatment of the soil, is necessary before this can be determined. With the cultures used in these experiments, however, the effective initial concentration in the soil appears to be in the range of 900 to 1025 p.p.m. This is a marked contrast to the lower concentrations

[4] Originated by C. J. King, Sacaton, Arizona.

[5] Sclerotia from soil culture 45-days-old.

TABLE 1.—*Results of analysis of cultures for ammonia, nitrate nitrogen, and moisture content*

Soil treatment[a]	P.P.M.—N as NH_3	P.P.M.—N as NO_3	Per cent moisture	Growth of fungus
Group 1[b]				
Check	17.1	15.1	.....	good
900 p.p.m. N as NH_3	396.4	248.7	.....	moderate
975 " " " "	407.8	264.3	.....	scant
Check	15.1	10.6	.....	good
Group 2[c]				
1025 p.p.m. N as NH_3[d]	498	499	30.0	none
1100 " " " "	547	542	35.4	none
1175 " " " "	628	522	36.0	none
1250 " " " "	697	517	34.6	none
1325 " " " "	788	483	30.5	none
Check	35	44	33.4	good

[a] Results expressed on oven-dry basis.

[b] All calculations based on 27 per cent moisture. Analysis made 26 days after treatment of soil.

[c] Analysis made 42 days after treatment of soil.

[d] Treatments were calculated on the basis of 30 per cent moisture. On the basis of the moisture content at date of analysis they were: 1025, 1146, 1230, 1294 and 1330 p.p.m., respectively.

of ammonia water previously referred to in this paper that were effective in killing root-rot mycelia and sclerotia when direct exposures were made in small tightly stoppered Erlenmeyer flasks.

BUREAU OF PLANT INDUSTRY,
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PHYTOPATHOLOGICAL NOTE

Pathogenic Strains in Ustilago nigra.—In the spring of 1935, ten collections of the black loose smut (*Ustilago nigra* Tapke)[1] of barley were used to inoculate 17 varieties. The smut collections had been increased on Alpha barley in the greenhouse during the winter of 1934-35 and were less than a month old and highly viable at the time of inoculation. Prior to inoculation, seed of the 17 barley varieties was treated by the modified hot-water treatment (5-hour presoak followed by 13-minute treatment at 52° C.) to insure freedom from any previous infestation of smut. The inoculated seed was sown in 5-foot rows in duplicate series in May, 1935, at Ithaca, New York, in cooperation with the New York (Cornell) Agricultural Experiment Station. In July the results shown in table 1 were obtained.

Table 1 shows that the varieties Himalaya (C. I. 1312), Lion (C. I. 923), and Nepal (C. I. 595) differentiated collection 186 from Wisconsin as a distinct pathogenic strain. The other 9 collections were pathogenically similar on this set of varieties.

In the fall of 1935 collections 185 and 186 were used again to inoculate smut-free seed of Himalaya, Lion, and Nepal. The inoculated seed was sown in November, 1935, in a greenhouse at the Arlington Experiment Farm, Rosslyn, Virginia. Collection 185 again failed to produce smut in Himalaya and Nepal but did produce 100 per cent smutted heads in Lion. Collection 186, on the other hand, produced 68.0 per cent smutted heads in Himalaya, 30.0 per cent in Nepal, and only 16.7 per cent in Lion. Each percentage is based on a total of approximately 100 heads.

The greenhouse results, therefore, confirmed the earlier field results in showing that pathogenic strains occur in *Ustilago nigra.*—V. F. TAPKE, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

[1] Tapke, V. F. A study of the cause of variability in response of barley loose smut to control through seed treatment with surface disinfectants. Jour. Agr. Research 25: 491–508. 1935.

TABLE 1.—*Percentages of smutted heads*[a] *in 17 varieties of barley grown at Ithaca, N. Y., in 1935 from seed inoculated with 10 collections of Ustilago nigra each from a different State*

Number and source of smut collection	Alpha C.I. 959	Club Mariout C.I. 261	Coast C.I. 2235	Excelsior violaceum C.I. 1248	Gatami C.I. 575	Hannchen C.I. 531	Hillsa C.I. 1604	Himalaya C.I. 1312	Hordeum deficiens C.I. 668–1	Hordeum intermedium C.I. 4377	Lion C.I. 923	Lyallpur C.I. 3403	Nepal C.I. 595	Odessa C.I. 934	Pannier C.I. 1330	Trebi C.I. 936	White Smyrna C.I. 910
125 New York	33.5	0	10.0	0	0	19.4	0	0	0	2.7	12.1	0	0	37.5	0	41.4	32.0
133 Missouri	20.2	1.1	11.6	0	0	28.5	0	0	0	0	16.4	0	0	57.6	0	43.8	16.5
143 Texas	22.6	0	3.6	0	0	25.7	0	0	0	0	13.8	0	0	57.5	0	29.8	15.6
176 Pennsylvania	32.2	0	7.8	0	0	16.9	0	0	0	0	11.0	0	0	60.1	0	24.5	22.2
179 Ohio	48.3	0	3.2	0	0	25.4	0	0	0	0	15.1	0	0	50.0	0	46.5	23.7
180 Iowa	29.0	0	4.8	0	0	30.1	0	0	0	0	18.4	0	0	60.1	0	43.8	20.6
181 North Dakota	27.0	0	3.9	0	0	33.9	0	0	0	0	35.4	0	0	47.0	0	47.9	25.1
184 Washington	42.0	0	7.6	0	0	31.2	0	0	0	0	30.2	0	0	70.2	0	44.3	34.1
185 Colorado	32.9	0	6.6	0	0	32.5	0	0	0	0	36.9	0	0	60.2	0	43.5	27.5
186 Wisconsin	23.4	0.9	3.4	0	0	15.6	0	16.8	0	0	2.5	0	32.0	47.9	0	32.7	10.0

[a] The total number of heads ranged from 146 to 563 in the different varieties.

BOOK REVIEW

Seifriz, William.—*Protoplasm.* 584 pp. *179 fig.* McGraw-Hill Book Co. New York and London, 1936. $6. (36s.)

Right at the outset, the reader of this interesting book on the physiology and morphology of protoplasm should be told that the title is merely a new (and very fitting) one for a book dealing with the principles of general physiology. After all, since the problems of cellular physiology are those of protoplasm, this should not be surprising. And a very instructive book it is, well adapted to the needs of advanced students who are interested in life *per se,* whether of plants or animals.

The first 15 of the book's 27 chapters deal primarily with the physics and chemistry of the protoplasmic stuff, in which field Seifriz is particularly at home. These chapters include a discussion of micrurgy, surface tension, viscosity, etc., with a summarizing chapter on "The structure of protoplasm and organic colloidal matter."

The next 7 chapters consider the cell and protoplasm in relation to the immediate environmental influences, and include discussions of permeability, acidity, electricity, radiant energy, and the rôle of water and salts in life processes.

The last section of the work includes a treatment of the products of protoplasm (carbohydrates, fats, proteins, hormones, etc.) and their interreactions with protoplasm. A final, very splendid chapter on "The origin of living matter," which permits the author to express his views on the philosophical problems of biology, closes the volume, not counting the bibliography (arranged according to chapters and authors) and the index (authors and subjects combined). In this last chapter Seifriz reviews the question of vitalism vs. mechanism and enrolls himself among the neovitalists (emergent evolutionists). While admitting that he has little hope that protoplasm will ever be manufactured *in vitro,* he urges that "the only safe method of procedure in science is the experimental and mechanistic one" and reminds us that "a severe mechanist may be more humanistic in his attitude towards the world than the most ardent vitalist." He then adds the significant statement that "the difficulty is that we have learned to control nature before we have learned to control ourselves."

In each chapter the author discusses the physical and chemical principles involved, and the book may thus serve as an excellent reference work for a statement of the fundamentals of biophysics and biochemistry. The first 15 chapters, which include the author's own field of research, are especially illuminating in this respect.

Although the book is on the whole well done and easily holds the reader's attention, the style, especially in the first chapters, while the author is

getting the feel of his pen, is in spots jerky, abrupt, disconnected, and even a trifle slovenly. On p. 19, for example, we read of "the Amoeba found in the sublingual spaces of our mouth," on p. 30 of "perpetual young tissue," and on p. 159 of "the initiation of the impetus to surface tension studies which has gone so far in biological reasoning." We also read in reference to tools used in micrurgy (p. 51) that "Certain of them can be bought, some of which are useful and timesaving"; that (p. 143) "The following diffusion constants are of several colloidal and crystalloidal substances"; and (p. 469) "A classified list, with examples, will convey an idea of the kind of substances that proteins are better than will a definition." The readers of the manuscript and proof, as well as the editors, failed in their duty here, or these sentences would not have escaped them. At times the idea expressed becomes almost naïve, as on p. 24 where we learn "It is the function rather than the reality of cell parts which troubles us most."

These defects in style frequently result in a distinct lack of clarity, as on p. 41 where we learn: "These latter swim about awhile as myxamoebae and then possibly fuse or individually grow into myxamoebae or plasmodia, which are the bodies of the plants."

Also on p. 177 we read: "The union between atoms established by the sharing of an electron is primary valence. Adsorption may be of this type and is then said to be *electronic*. It is distinguished from primary valence in that . . ." Is adsorption primary valence or not? Now you see it; now you don't.

Again on p. 178 we find: "More numerous are those looser bonds, which go under the name of *coordination numbers*. These latter are significant in adsorption. Every atom in a crystal of sodium chloride is surrounded by six of the opposite kind." Opposite kind of what?

The story is told that when Jacques Loeb was once criticised for a lack of clarity, he replied: "Arrhenius will understand." It doesn't always take an Arrhenius to understand what Seifriz means, but clarity could have helped even a Jacques Loeb.

The reader who likes a book of science to possess a certain uniformity and regularity (which we like to think Nature approves of) will regret to see names of genera and species only occasionally italicized (p. 450); generally they are not. Also he will wonder why the same name (*e.g.* Myxomycetes) sometimes (p. 40) is capitalized and sometimes not (p. 7) This, of course, is assuming that some virtue may lie in uniformity and consistency.

Also (as in most books) there are a few errors that can be ascribed directly to the printer and proof-reader, but such errors are not of especial importance; they are only annoying.

But not all the "errors," unfortunately, can be placed on the shoulders of proof-readers. Some of these, to be sure, are subject to differences of opinion. The present reviewer seriously questions whether viable Nelumbo seed 200 years old have ever been found (p. 6). Also it is possible that *Oxalis stricta* and *Rumex acetosella* will grow in the laboratory at pH 8, but it is doubtful whether they are found growing luxuriantly in nature at this pH. The old "tradition among farmers" (p. 323) that these plants indicate sour soil may still continue to be fostered, even though it is true that such soils also are frequently sterile. Most farmers know, however, that in a majority of cases liming soils where sorrel and "sour-grass" grow will result in marked improvement.

Again, on p. 245, we read that "the red blood cell of amphibians is 'simply a sac' with 'no visible framework' of structure." In view of the presence of the nucleus mentioned, this seems difficult to understand.

Also on p. 531 the idea is expressed that small cells don't have enough room "for any but simple chemical reactions." Even 200 molecules, if of different kinds, would seem to give plenty of opportunity for complicated reactions, and there is little evidence to indicate that the complexity of cell reactions is associated with size of cell. Quite the contrary, a small, independent bacterial cell may be the seat of reactions much more complicated than those in a much larger tissue cell.

In several places lack of proper digestion of the material seems a bit obvious. On p. 220 we learn that high metabolic activity is associated with low protoplasmic viscosity (called unfortunately "consistency" here and there), but in the next paragraph an example is given of mitosis, during the early stages of which there is an increase in viscosity. Surely, during mitosis, if ever, cells are active. Again in the excellent chapter on elasticity we are told (and correctly) that elasticity must not be confused with ductility or extensibility; and yet, on p. 232 the author himself (by aid of the figure) says he is demonstrating elasticity when he is really demonstrating ductility and extensibility. Similarly, on p. 420, we learn (correctly, I believe) that diabetes is associated with acidity, but on the next page we are told that it is alkalinity that accompanies diabetes. Again, on p. 419, we are told that "a fasting animal may lose all its fat and half its protein but not more than one-tenth of its water"; but two pages farther on we read that "during starvation, the largest loss in body substance is water."

In not all his errors, however, is the author so doubtfully inconsistent. Not all cells of mushrooms are binucleate, as is distinctly implied (p. 17); the hollow-ground slide is at the *left* in Fig. 52; in E, Fig. 91 no continuous spiral is evident, as told us on p. 153; an exothermic reaction is not one that "proceeds without elimination of heat" (p. 458); and chlorophyllase is not

the enzyme responsible for forming sugar from CO_2 and H_2O (508), to cite some of the most obvious errors.

Whether there are errors of *omission* will depend much on the individual reader, but, surely, any list of essential elements and their rôles in plant and animal cells should include under Mg its function in the chlorophyll molecule.

I hope the readers of this review will not think this is a poor book. It is, on the contrary, a very good and inspiring book and one that has been needed in English for some time. If Homer nodded, a physiologist should be allowed a wink or two. The errors cited can easily be straightened out in the 2nd edition, which the publishers seem to promise us with their "First Edition" on the title page (or is this merely a guarantee to bibliophiles and bibliopolists?).

Students of phytopathology are realizing more and more the relations between their subject and cellular physiology. While this book does not relate directly to pathology, it bears many allusions thereto, and the pathologist who reads here will find much to interest him. Wise is the man who knows always what he *ought* to be interested in. But, pathologist or not, this work should find a place on the shelves of all who would like to know what makes our wheels go 'round and to know more of the interrelations between chemistry, physics, and biology.—ORAN RABER.

REPORT OF THE TWENTIETH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The twentieth annual meeting of the Pacific Division of The American Phytopathological Society was held at the University of Washington, Seattle, June 16 to 20, 1936, in affiliation with the Pacific Division of the American Association for the Advancement of Science. Three half-day sessions were held and 24 papers were presented.

The sessions were well attended considering the fact that the place of meeting was in the extreme Northwest. About 30 members and visitors registered, 2 from as far away as Arizona.

At a business meeting the following officers were elected:

President—B. A. RUDOLPH, University of California, Deciduous Fruit Station, San Jose, Calif.

Vice President—C. W. BENNETT, United States Department of Agriculture, Riverside, Calif.

Secretary-Treasurer—L. D. LEACH, University of California, Davis, Calif.

Councilor—J. W. HOTSON, University of Washington, Seattle, Washington.

The papers covered a variety of subjects. The majority of them were of a strictly pathological nature, but several mycological papers were presented. The pathological subjects discussed included viroses and vectors, fungus diseases of fruits, cereals, and trees, and experiments with various fungicides in controlling plant diseases.

H. P. Severin and J. C. Freitag discussed the transmission of western celery mosaic by aphids and the properties of the virus, also the aphid vectors of celery calico. A. B. Hatch discussed mycorrhizal fungi, calling attention to the greatly increased absorbing surface on pine roots induced by these fungi and the fact that trees cannot thrive in infertile soil in their absence. William Newton described the effects on tomato of a particularly virulent strain of the "potato X" virus. L. J. Klotz described an injury to citrus fruits caused by water. H. S. Fawcett and L. J. Klotz reported a new species of Candelospora causing decay in citrus fruits. G. A. Huber made a progress report on the control of narcissus and bulbous iris leaf diseases by Bordeaux-penetrol spray, and on the spread and adhesiveness of this spray material. This combination proved the most satisfactory of any materials tried. J. Kienholz and Leroy Childs conducted trials of various copper sprays for peas and apple scab, considering both effectiveness and

tendency to cause injury. A 2–2–2–50 combination of copper phosphate, lime, and bentonite proved best. R. B. Streets discussed attempts to diagnose cotton root rot in soils by screening out the sclerotia, and indicated the apparent usefulness of heavy applications of ammonium sulphate in aiding recovery of fruit and nut trees from root rot. R. Sprague reported on foot rots of cereals in certain coastal valleys of Oregon, and also on some mycological aspects of species of Mastigosporium on Gramineae. G. W. Fischer found that certain smut spores taken from herbarium specimens were viable after periods ranging up to twenty-five years. S. M. Zeller outlined studies on bark necrosis and fruit pit of pear, reported simultaneous infection of strawberry by crinkle and yellows, and described a new genus of gasteromycetes. Karl D. Butler described a disease of the inflorescence of the date palm caused by a Helminthosporium, and reported studies on ash tree cankers. Several papers, chiefly of mycological interest, were presented as follows: J. W. Hotson, A new species of Arachniotus isolated from contaminated milk; R. H. Tschudy, Cultural studies of the genus Chaetomium; and studies of several groups of fungi as they occur in western Washington, namely, operculate Discomycetes by Leon C. Snyder; the genus Inocybe by D. E. Stuntz; Polypores by J. R. Roberts; and Hydnums by W. M. Lanphere.

The University of Washington proved a very hospitable host. The campus was beautiful, and everything was done for the comfort and convenience of the visitors. One of the high lights of the meeting was a dinner for all biologists on Thursday evening, which was attended by several hundred, including many pathologists.

The 1937 meeting will be held the third week of June in Denver, Colorado, as a joint meeting with the National Association and the Southwestern Division.

L. D. LEACH,
Secretary-Treasurer.